

## I U C L I D

## Data Set

Existing Chemical : ID: 106-93-4  
CAS No. : 106-93-4  
EINECS Name : 1,2-dibromoethane  
EINECS No. : 203-444-5  
TSCA Name : Ethane, 1,2-dibromo-  
Molecular Formula : C<sub>2</sub>H<sub>4</sub>Br<sub>2</sub>

Producer Related Part  
Company : GREAT LAKES CHEMICAL CORPORATION  
Creation date : 18.12.2001

Substance Related Part  
Company : GREAT LAKES CHEMICAL CORPORATION  
Creation date : 18.12.2001

Memo :

Printing date : 28.12.2001  
Revision date :  
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Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

# 1. General Information

Id 106-93-4  
Date 28.12.2001

## 1.0.1 OECD AND COMPANY INFORMATION

## 1.0.2 LOCATION OF PRODUCTION SITE

## 1.0.3 IDENTITY OF RECIPIENTS

## 1.1 GENERAL SUBSTANCE INFORMATION

### 1.1.0 DETAILS ON TEMPLATE

### 1.1.1 SPECTRA

## 1.2 SYNONYMS

## 1.3 IMPURITIES

## 1.4 ADDITIVES

## 1.5 QUANTITY

### 1.6.1 LABELLING

### 1.6.2 CLASSIFICATION

## 1.7 USE PATTERN

### 1.7.1 TECHNOLOGY PRODUCTION/USE

## 1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

## 1.9 SOURCE OF EXPOSURE

### 1.10.1 RECOMMENDATIONS/PRECAUTIONARY MEASURES

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1.10.2 EMERGENCY MEASURES

1.11 PACKAGING

1.12 POSSIB. OF RENDERING SUBST. HARMLESS

1.13 STATEMENTS CONCERNING WASTE

1.14.1 WATER POLLUTION

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1.14.3 AIR POLLUTION

1.15 ADDITIONAL REMARKS

1.16 LAST LITERATURE SEARCH

1.17 REVIEWS

1.18 LISTINGS E.G. CHEMICAL INVENTORIES

## 2. Physico-Chemical Data

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### 2.1 MELTING POINT

Value : = 9.3 - 9.8 ° C  
Reliability : (2) valid with restrictions  
19.12.2001

(15) (18) (31)

### 2.2 BOILING POINT

Value : = 131 - 131.4 ° C at  
Reliability : (2) valid with restrictions  
19.12.2001

(12) (18) (31)

### 2.3 DENSITY

#### 2.3.1 GRANULOMETRY

### 2.4 VAPOUR PRESSURE

Result : 11.7 mmHg @ 25 degrees C  
Reliability : (2) valid with restrictions  
20.12.2001

(15)

Result : 14.7 mmHg @ 20 degrees C  
Reliability : (2) valid with restrictions  
20.12.2001

(46)

Result : 23.2 mmHg @ 30 degrees C  
Reliability : (2) valid with restrictions  
20.12.2001

(31)

### 2.5 PARTITION COEFFICIENT

Log pow : = 1.93 - 2.13 at ° C  
Reliability : (2) valid with restrictions  
19.12.2001

(15) (32) (46)

#### 2.6.1 WATER SOLUBILITY

Value : = 4.04 g/l at 20 ° C  
Qualitative :  
Pka : at 25 ° C  
PH : at and ° C  
19.12.2001

(46)

Value : = 4.3 g/l at 30 ° C  
Qualitative :  
Pka : at 25 ° C  
PH : at and ° C  
Remark : "slightly soluble"  
Reliability : (2) valid with restrictions

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(18) (27)

**Result** : "slightly soluble"  
**Reliability** : (2) valid with restrictions  
19.12.2001

(12)

### 2.6.2 SURFACE TENSION

### 2.7 FLASH POINT

### 2.8 AUTO FLAMMABILITY

### 2.9 FLAMMABILITY

### 2.10 EXPLOSIVE PROPERTIES

### 2.11 OXIDIZING PROPERTIES

### 2.12 ADDITIONAL REMARKS

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#### 3.1.1 PHOTODEGRADATION

Type : air  
Light source :  
Light spect. : nm  
Rel. intensity : based on Intensity of Sunlight  
Deg. Product :  
Method :  
Year : 1976  
GLP :  
Test substance :  
Method : STUDY OBJECTIVES: Measure the absolute rate constants for the reaction of OH radicals with ethane and twelve fluorine, chlorine, and bromine substituted ethane compounds

RECOGNIZED METHOD: No

GLP: Pre-GLP

TEST SUBSTANCE: All substances tested were 99+% pure. The analyses were provided by the manufacturers.

CONCENTRATION OF TEST SUBSTANCES: Typical concentrations were  $10^9$  -  $10^{11}$  molecules/cubic cm for OH,  $8 \times 10^{12}$  -  $6 \times 10^{15}$  molecules/cubic cm for reactant molecules, and  $2.6 \times 10^{16}$  -  $2.6 \times 10^{17}$  molecules/cubic cm for helium carrier gas.

EXPOSURE PERIOD: Not applicable

TEST PROCEDURE: The apparatus was a conventional discharge-flow system in which OH radicals are generated in a helium carrier gas stream by the fast reaction of H with nitrous oxide. The gas temperature was 296 degrees K. Hydroxyl radicals are detected with a laser magnetic resonance spectrometer.

Result : RESULTS: The average rate constant for EDB from 19 separate measurements was:  $250(18) \pm 55$ , or roughly >1 month (tropospheric life time). Ethylene dibromide degrades in the atmosphere by reacting with photochemically-produced hydroxyl radicals. Due to very slight absorption above 260 nm, direct photolysis occurs very slowly, if at all. The authors conclude that this suggests a very small fraction of the EDB released into the troposphere will reach the stratosphere. Comparisons were made to chloro-fluorocarbons with predicted lifetimes of 10 years and therefore calculated to last long enough to reach the stratosphere.

Reliability : (4) not assignable  
19.12.2001

(22)

Type : water  
Light source : other: 240 W Hanovia medium-pressure mercury lamp  
Light spect. : nm  
Rel. intensity : based on Intensity of Sunlight  
Conc. of subst. : at 22 degree C  
Deg. Product :  
Method :  
Year : 1988  
GLP :  
Test substance : other TS: Ethylene Dibromide (EDB) was obtained from Matheson, Coleman and Bell and was used without further purification  
Method : STUDY OBJECTIVES: The objective of the study was to determine the effect of irradiation on the rate of hydrolysis.

**RECOGNIZED METHOD:** The authors did not indicate they followed a pre-established protocol or guideline. The reference OECD guideline is OECD 111.

**GLP:** Pre-GLP.

**TEST SUBSTANCE:** Ethylene Dibromide (EDB) was obtained from Matheson, Coleman and Bell and was used without further purification

**Purity:** Purity of the starting material was not given in the report. The substance did exhibit a single peak upon gas chromatography and showed correct mass spectrum.

**OTHER MATERIALS:** Ethylene oxide was obtained from Matheson, and analytical grade ethylene glycol was obtained from Mallenckrodt and employed without further purification. Bromoethanol was obtained from Eastman Kodak and distilled (bp 55-56 degrees C @20 mmHg) before use. All substances exhibited a single peak upon gas chromatography and showed correct mass spectra. Potassium ferrioxalate was prepared according to procedure of Parker (1953). Water was deionized and glass distilled.

**CONTROLS:** As a non-irradiated control, Br ion was determined in a 0.001 M solution of EDB that had been standing in a sealed flask for 0.77 year. The concentration of Br ion was 0.00032 M. This corresponds to a half-life of 16 years.

**CONCENTRATIONS OF TEST SUBSTANCES:** Starting concentrations for all substrates were stated to be in the range of 0.009-0.09 M. The study did not state how the concentrations of the starting material were determined.

**EXPOSURE PERIOD:** Exposure to irradiation varied depending upon the compound being irradiated. EDB: 2hr.; bromoethanol: 3hr.; ethylene oxide: 3 hr.; and potassium ferrioxalate: 15 minutes.

**EQUIPMENT:** A 700 mL tube-shaped reactor was fitted with serum-capped stopcocks for gas and solution removal, manometer, and magnetic stirring bar. The light well was a concentric, water-jacketed quartz finger. Solution levels were 2-3 cm above the top of the tubular 450-W Hanovia medium-pressure mercury lamp.

**TEST PROCEDURE:** The degradation of solutions of ethylene dibromide, bromoethanol, and ethylene oxide under direct irradiation using a 240 W Hanovia medium-pressure mercury lamp. The reactor was charged with 600 mL of an aqueous solution in air, and placed in a water bath at 22 degrees C. Aliquots of the reaction solution were taken with time. The concentration of Br ion was determined potentiometrically. Ethylene Dibromide, (138 degrees C, 8.0 min), bromoethanol (138 degrees C, 4.0 min), and ethylene oxide (90 degrees C, 3.0 min) were determined from 1-mL reaction aliquots by direct flame ionization gas chromatography, and quantified. The nature of the photoproducts was confirmed by GC-MS.

Relative degradation rates of EDB and ferrioxalate were also assessed under sunlight irradiation. Stock solutions of EDB and ferrioxalate were placed in sealed quartz tubes, placed on the roof and sampled for Br ion and Fe<sup>2+</sup>.

**STATISTICAL METHODS:** For Ethylene Dibromide and bromoethanol, the rate of reaction was assessed from linear first-order plots of the disappearance of EDB with time. Reproducibility was  $\pm 10\%$  and  $\pm 5\%$  for

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#### Result

EDB and Bromoethanol, respectively.  
: RESULTS: A quantitative reaction sequence for the photolytic process was determined. The first step is hydrolysis to bromoethanol with a half-life of 7.6 minutes. This is followed by a slower hydroxylation step of bromoethanol to ethylene oxide with a half-life of 64 minutes. The final hydrolysis of ethylene oxide to ethylene glycol is not enhanced by light (unlike the first two steps) with a half-life at pH 7 of 10 days.

The relative rates of the light enhanced hydrolysis and hydroxylation steps compared to the rate of reduction of potassium ferrioxalate are 32 for EDB and 3.8 for bromoethanol, respectively. With sunlight irradiation EDB reacts 2.7 times faster than ferrioxalate. An estimate of the rate for Br ion release from EDB based on 53 days (and nights) of exposure to roof sunlight, (10% conversion) corresponds to a half-life of approximately 380 days. No direct photolysis of any consequence is expected under environmental conditions.

Reliability  
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: (2) valid with restrictions

(4)

#### 3.1.2 STABILITY IN WATER

##### Type

: abiotic

##### t1/2 pH4

: at degree C

##### t1/2 pH7

: at degree C

##### t1/2 pH9

: at degree C

##### Deg. Product

:

##### Method

:

##### Year

: 1984

##### GLP

:

##### Test substance

: other TS: Ethylene dibromide

##### Method

: STUDY OBJECTIVE: This study was designed to determine hydrolysis rates of ethylene dibromide (EDB) at two temperatures, each with three pH regiments. These six test regimes, all conducted in darkness, were expected to provide estimates of EDB half-life and the applicable rate law.

RECOGNIZED METHOD: Comparable to OECD guideline 111.

GLP: Pre-GLP.

TEST MATERIAL: Ethylene dibromide (EDB). Solutions of EDB in aqueous buffers were prepared as follows. The EDB was weighed in a small glass thimble and was dropped in a 1 Liter volumetric flask filled to the mark with the corresponding buffer. A magnetic stirrer bar was added; the flask was then stoppered and agitated overnight or until the glass thimble was broken into small pieces and the EDB completely dissolved. The water and glassware were sterilized in an autoclave. Purity and the source supplier of the EDB was not given in the report

CONTROLS: Several ampules of standard EDB solution (96 ppm) in distilled water were sealed and stored in a refrigerator at 4°C.

CONCENTRATIONS OF TEST SUBSTANCES: Three solutions were prepared one in the buffer of pH 5 (EDB concentration of 95.6 ppm); the second in the buffer of pH 7 (EDB concentration of 88.8 ppm); and the third in the buffer of pH 9 (EDB concentration of 85.1 ppm). The report does not give the composition of the buffers or whether the buffers were sterilized prior to using them in the study.

The EDB concentration in the buffer solutions was calculated by comparing the area of the sample peak to the area of the reference standard of known



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concentration.

**STATISTICAL METHODS:** The half-life for the decomposition of EDB was calculated assuming the reaction rate was between zero order kinetics and first order kinetics, owing to the nature of the molecule.

**Zero Order:** A plot of concentration vs. time was made and the half-life determined from this plot.

**First Order:** Assuming the hydrolysis of EDB follows first order reaction, the half-life was calculated from the kinetic models.

**EXPOSURE PERIOD:** Samples were taken on day 0, 30, 60, 95, and 140 days.

**EQUIPMENT:** The analysis for volatile organic byproducts was done using a purge-and-trap module (Tekmar) interfaced with a Finnigan GC/MS (OWA-1000).

**TEST PROCEDURE:** Ampules of each buffer solution were placed in incubators maintained at 25 or 45 degrees C. One ampule for each pH and temperature (total of six) was withdrawn from the incubators and analyzed for EDB concentration on day 0, 30, 60, 95, and 140 days. Each ampule was broken and 0.4 mL samples were withdrawn with a microsyringe for chromatographic analysis. Six replicate analyses were performed from each ampule and the mean of all six analyses was calculated and used as the concentration at the day.

The analysis for volatile organic byproducts identified only ethylene bromide. Its concentration could not be assayed due to losses of evaporation upon breaking the sample ampule and to unknown trapping efficiency of the Tenax trap for ethylene bromide.

#### Result

: RESULTS:

·The rate of hydrolysis of EDB is very slow at 25 degrees C, but increases as the pH decreased from 9 to 5.

·The rate of hydrolysis at 45 degrees C is several times faster than at 25 degrees C at all three pH's, and the kinetic order approaches one. At 45 degrees C the rate increased with increasing pH.

·The primary reaction of EDB decomposition appears to be dehydrohalogenation, at least at 45°C and pH 9.

·The product of debromination, ethylene, was not detected.

·The percentage of EDB hydrolyzed after 140 days was 0-13% (@ 25 degrees) and 33-42% (@ 45 degrees).

**Reliability**  
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: (2) valid with restrictions

(17)

**Type**

: abiotic

**t1/2 pH4**

: at degree C

**t1/2 pH7**

: at degree C

**t1/2 pH9**

: at degree C

**Deg. Product**

:

**Method**

:

**Year**

: 1986

**GLP**

:

**Test substance**

:

**Method**

: **STUDY OBJECTIVES:** This study reports on the reactivity of some mono- and dibromo-alkanes in aqueous buffers. Hydrolysis and elimination

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reactions were considered. The available data on the reactivity of alkyl bromides in water were analyzed with respect to Taft's linear free energy relationship (LFER).

RECOGNIZED METHOD: No. Comparable to OECD Guideline 111 for hydrolysis.

GLP: Pre-GLP.

TEST SUBSTANCE: 1,2-DBP, 1,2,3-tribromopropane (1,2,3-TBP), 1,3-DPB, EDB, 1-bromo-3-phenylpropane (BPP), and 1-bromo-n-heptane (BH). They were the highest purity available and used as received.

Stock solutions of the organics were prepared in methanol. Pentane was used as the extractant.

OTHER MATERIALS: Reagents for the buffer solutions were obtained from Baker Chemical Company. Solutions were adjusted to the correct pH at the reaction temperature with either dilute HCl or NaOH. The ionic strength of the final solutions was 0.1 M. The pH was measured at the experimental temperature by using a glass electrode.

CONTROLS: An internal standard (IS) was added to the pentane before extraction. 1,3-DBP was used as the IS For 1,2-DBP, and vice versa. Tetrachloroethylene was used as the IS for EDB. Mass spectra were compared with spectra from the National Bureau of Standards library for product identification.

CONCENTRATIONS OF TEST SUBSTANCES: Not given

EQUIPMENT: An electron capture detector (ECD) was used for 1,2-DBP, 1,3-DBP, and EDB, and a flame ionization detector (FID) was used for BH and BPP. Peak-area integration was carried out with a Model 4000 data system (Spectra Physics). Pentane-extractable reaction products were analyzed by GC/MS.

TEST PROCEDURE: The kinetic experiments were performed in flame-sealed glass ampules. Ten milliliters of each buffer solution was pipetted into glass ampules, which were subsequently spiked with the alkyl bromide solution (10 mg/l) and immediately sealed with a propane flame. The ampules were immersed in a temperature-controlled water bath, the rates studied in the 45 - 90degrees C temperature range, and removed at pre-selected time intervals after the start of the experiment. These were added to volumetric flasks containing pentane, stoppered tightly and shaken vigorously by hand for 3 minutes.

Two microliters of the pentane extract was injected onto a 15-m Durabond DB-5 thick-film capillary column using a 200 degrees C Grob injector and quantified. Quantification was based upon peak-area integration with a Model 4000 data system. The internal standard was added to the pentane before extraction.

Pentane-extractable reaction products were analyzed by GC/MS for product identification.

The fraction of an organic in the gas phase was estimated from its Henry's constant, H, the liquid-phase volume and vapor-phase volume (1). H was estimated from vapor pressure and solubility data (2). The 20 degree C H constants of the compounds evaluated ranged from 0.25 atm×L/mol for 1,3-DBP to 6.6 atm×L/mol for BH. The vapor to liquid ratios at 20 degrees C were estimated to range from 1 x 10E-4 for 1,3-DBP to 3 X 10E-3 for BH. Such small gaseous fraction (<0.3%) would not normally affect the

determination of aqueous reaction rates. However, Munz (3) found that H increased by a factor of approximately 2 for a 15 degree C temperature increase within the range 10 - 30 degrees C. Assuming the H doubles every 15 degrees C over the entire temperature range (20 - 95 degrees C), H at 95 degrees C increases by a factor of 32. Hence, the gas to liquid ratio of BH at 50 degrees C would be ~1% and at 95 degrees C, it would increase to ~10%. This estimation was taken into account, particularly for compounds with a very low water solubility such as BH.

STATISTICAL METHODS: The Arrhenius equation was used to extrapolate the reaction rate constant measured at elevated temperatures to 95 degrees C. The statistical uncertainties of the activation energy and observed first order rate constant of overall hydrolysis at 25 degrees C were estimated according to procedures of Lindren (4).

Citations used in Text:

1. Burlinson, N. E.; Lee, L. A.; Rosenblatt, D. H., Environ. Sci. Technol., 1982, 16,627-632.
2. Thomas, R. G., In Handbook of Chemical Property Estimation Hanbook; :Lyman, W. J.; Reehl, W. F.; Rosenblatt, D. H., Eds.; McGraw-Hill: New York, Ny 1982; pp 15-1 - 15-34.
3. Munz, C., Ph. D. Dissertation, Stanford University, Stanford, CA, 1985

4. Lindgren, B. W., Statistical Theory, MacMillan, New York, NY, 1976, pp533-546.

Result

- : RESULTS: BPP and BH reacted to the corresponding alcohols with no elimination products detected by pentane extraction and GC/MS. EDB, 1,2-DBP, and 1,2,3-TBP reacted to form various bromoalkene isomers: EDB yielded vinyl bromide. Dehydrobromination has been reported to be the major transformation for DBCP in buffered aqueous solutions (1).

Pseudo-first order reaction (i.e., disappearance) rates were observed. In general, all disappearance rates were pH-independent at a pH range of 7 - 9. The reaction rates and half-lives extrapolated to 25 degrees C were calculated by using the Arrhenius relationship. From this it was determined that the half-life for EDB was 2.5 years.

In general the estimated half-lives of alkyl bromides increased with the degree of halogenation. The data evaluated by linear free energy relationships indicate a general decrease in reactivity, i.e., an increase in half-lives, with increase in the inductive constant. Any attempt to correlate the data presented to groundwater conditions should be made with caution because study conditions used solutions of relatively high ionic strength (0.1 M), higher than what is typical for fresh waters (<0.01M) (5), and at temperatures 20-70 degrees C above typical environmental temperatures.

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- : (4) not assignable

(39)

3.1.3 STABILITY IN SOIL

3.2 MONITORING DATA

- Type of measurement : other: Modeling (bioconcentration)  
Medium : soil  
Method :  
Concentration :  
Method : STUDY OBJECTIVES: To predict (through modeling) soil sorption coefficients and bioconcentration factors for compounds where these values are not known but water solubility is known.

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RECOGNIZED METHOD: No

GLP: Not applicable

TEST SUBSTANCE: Not applicable

OTHER MATERIALS: Not applicable

CONCENTRATIONS OF TEST SUBSTANCES: Not applicable

EXPOSURE PERIOD: Not applicable

EQUIPMENT: Not applicable

TEST PROCEDURE: This report uses certain equations developed by Kenaga and Goring to predict soil sorption coefficients and bioconcentration factors for compounds where these values are not known but water solubility is known.

The equations used in this study, taken from Kenaga and Goring (1) are shown below.

- a.  $\text{Log BCF}^* = 2.791 - 0.564 (\log \text{WS}) \pm 1.99 \text{ orders of magnitude (OM) (95\% confidence limit) from the calculated value. WS units are ppm.}$
- b.  $\text{Log BCF}^* = -1.576 + 1.119(\log \text{Koc}) \pm 1.95 \text{ OM (95\% confidence limit) from the calculated value.}$

\*BCF values are calculated from equations for flowing water systems (f) rather than from the terrestrial-aquatic systems (t) shown by Kenaga and Goring (1978).

BCF (f) value average almost one order of magnitude higher than BCF(t) values. The equation used for prediction of soil sorption coefficient values was as follows:

- c.  $\text{Log Koc} = 3.64 - 0.55 (\log \text{WS}) \pm 1.23 \text{ OM (95\% confidence limits) from calculated value.}$

Water solubility data were obtained from the summaries of Kenaga and Goring (1) and the Pesticide Manual of Martin and Worthing (2). Soil sorption data were obtained from the summary of Kenaga and Goring (3). Two predictive values for BCF (derived from WS and Kos equations) were given for comparative purposes.

The validity of these equations was questioned for use with compounds that do not penetrate tissue, are rapidly degraded or rapidly lost from soil or water, are rapidly metabolized by living organisms, or are ionic and bound strongly to soil by electrostatic interaction. Exceptions and limitations in the use of these equations for predictive purposes were addressed.

Citations used in Text:

- 1. Reference not given in paper
- 2. Martin, H. and Worthing, C.R., (1977), Pesticide Manual 5th ed. British Crop Protection Council, Worcestershire, England.
- 3. Kenaga, E. E., and Goring, C. A. I. (1980). Relationship Between Water Solubility, Soil Sorption, Octanol-Water partitioning, and Bioconcentration of Chemicals in Biota. In Aquatic Toxicology ASTM STP 707, (J. C. Eaton, P. R. Parrish, and A. C. Hendricks, Eds.), American Society for Testing and Materials, in press. ( In the paper the reference indicates Kenaga and Goring 1978)

Reliability

: (4) not assignable

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**Type of measurement** :  
**Medium** : soil  
**Method** : Critically examines different methodologies for determining residual EDB in soil.  
**Concentration** :  
**Method** : STUDY TYPE: Analytical Method Development for Residual EDB

**STUDY OBJECTIVES:** To critically examine several likely methods for determining residual EDB in the soil leading to the development of a satisfactory method.

**RECOGNIZED METHOD:** The method developed is applicable to aerobic and anaerobic transformation in soil (OECD 307), but is an analytical method as opposed to an analysis of degradation.

**GLP:** Pre-GLP

**TEST SUBSTANCE:** Radiolabeled EDB (204 x 10E7 Bq/mmol) Obtained from Amersham

**CONTROLS:** The EDB was identified in extracts of the field soils by GC/MS by comparison with analytical compound.

**CONCENTRATION OF TEST SUBSTANCES:** Not Applicable

**EXPOSURE PERIOD:** Not Applicable

**EQUIPMENT:** Hewlett Packard 5988A GC/MS. The mass spectrometer was operated in the electron impact mode (70eV). These signals represent the loss of Br from the parent ion. The limit of detection was 10 pg EDB.

Hewlett Packard 7675A purge and trap unit with N2 gas for different periods of time either at 40 or 80 degrees C. The EDB was analyzed with a Hewlett Packard 5840 gas chromatograph using a stainless steel column and flame ionization detector.

For thermal desorption, hexane was analyzed for EDB by GC isothermally at 80 degrees C using a 2 m by 4 mm o.d. column of 155 OV17 on Chromosorb WHP (80/100 mesh) and a Ni(63) electron capture detector with a 40 mL/min flow of 5% methane in argon. The detection limit was 1.5 pg EDB (0.3 mg/L EDB in hexane).

**TEST PROCEDURE:** For purge and trap, a 1 gram soil sample was analyzed for EDB by gas chromatography using a flame ionization detector.

Thermal desorption removal of EDB at 100 to 200 degrees C was carried out by passing a stream of nitrogen gas at 20 mL/min through a soil column. The detection limit was 1.5 pg EDB.

In the solvent extraction study, the soil was extracted with methanol, acetone, acetonitrile, hexane, or hexane-water mixtures at different temperatures.

Sonication-extraction was carried out on 30 g soil in 100 mL methanol in a 400-mL beaker.

**Result** : **RESULTS:** The authors compared several methods for determination of EDB in the field samples: purge and trap, thermal desorption, and various solvent extraction methods. The only satisfactory one was a solvent extraction technique.

The purge and trap method, as prescribed by EPA Method 8240 for estimating VOCs in soils, removed only small amounts of EDB. Purging at higher temperature and longer periods of time increased the release of EDB. Nonetheless the amount released was <11% of the total found by the recommended solvent extraction method.

In the thermal desorption method, essentially no EDB was desorbed from the field-contaminated soil over the temperature range studied.

These results demonstrated that thermal desorption methods are not suitable for determining residual EDB in soils.

A comparison of the ability of different organic solvents to extract EDB showed that two 24 hours extraction with methanol at 75 degrees C recovered essentially 100% of the residual EDB.

The use of Sonication-extraction method (1) with methanol for 1.5 minutes total Sonication time recovered only 12%, based on total EDB removed by the recommended method below.

The study results indicate the following procedure is the best method for removal of EDB residual from soil. Weigh 5 g of soil in a 40-mL glass screw-cap vial, add 25 mL methanol, and cap firmly. Invert the vial and mark the level of liquid as a reference for determining any leak during heating. Place the vial inverted in an incubator at 75 degrees C for 24 hour. After 24 hours, allow the vial to cool. After cooling, check the level of liquid and discard any vial where loss in volume is indicated. Centrifuge (1500 rpm for 10 min) and transfer the supernatant into a 200-mL flask. Resuspend the soil in 25 mL methanol, centrifuge, and transfer the supernatant to the flask. Add 30 mL hexane and 100 mL distilled water to the combined methanol extracts. Shake vigorously for 30 seconds and allow the phases to separate. The volume of the upper hexane layer is not appreciably different from the volume of hexane added. Analyze the hexane layer by GC and multiply the calculated total EDB by 1.13 to account for EDB left in the water-methanol layer. The detection limit is 1.8 mg/kg using a ratio of 5 g soil to 25 mL methanol.

STATISTICAL METHODS: None

Citation used in Text:

1. U.S. Environmental Protection Agency, 1982, Test methods for analysis of solid waste: Physical/chemical methods. SW-846 2nd ed., Office of Solid Waste and Emergency Response, Washington, DC.

Reliability

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: (2) valid with restrictions

(5)

#### 3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

#### 3.3.2 DISTRIBUTION

#### 3.4 MODE OF DEGRADATION IN ACTUAL USE

#### 3.5 BIODEGRADATION

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Type : aerobic  
Inoculum :  
Deg. Product :  
Method : other: Comparable to OECD Guideline 307, Aerobic Transformation in Soil.  
Year : 1986  
GLP :  
Test substance :  
Method : STUDY OBJECTIVES: This report reexamines biodegradation of EDB by soil microorganisms.

RECOGNIZED METHOD: No. Comparable to OECD Guideline 307, Aerobic Transformation in Soil.

GLP: Pre-GLP.

TEST SUBSTANCE: 1,2-dibromoethane (ethylene dibromide, EDB); Purity: not given.

Radiolabeled (C14) EDB (23 mCi/mmol; 98% radiochemical purity) was obtained from New England Nuclear Corp., Boston, Massachusetts.

OTHER MATERIALS: Samples were collected from a site that overlies an aquifer (10 to 20 m of sandy soil above bedrock), which discharges into a shallow stream. The stream and several test and production wells had been contaminated with EDB in the range of 0.1 to 11 mg/L for at least 1 year prior to sampling for this study.

Sampled sites collected represent extremes of organic compound content and microbial activity. The first soil sample (S1) was composed of organic carbon-poor (0.24% organic carbon), medium-to-coarse sand taken from a streambed which was aerobic in its native state. Experiments were conducted with a 3:2 mixture (dry wt/vol) of solid material and accompanying stream water. The second soil sample (S2) was composed of organic carbon-rich (14% organic carbon), muddy soil from an area partially anaerobic in its native state. Levels of EDB in this site were 4.2 and 8.6 mg per kg.

CONTROLS Autoclaved controls of S1, S2, stream water, and distilled water were run concurrently.

CONCENTRATIONS OF TEST SUBSTANCES: Incubations were carried out at either 6 to 8 ppb (mg/L) or 15 to 18 ppm (mg/L) of EDB.

EXPOSURE PERIOD: Exposure times varied from 0 to 35 weeks.

TEST PROCEDURE: S1 and S2 were added to flasks, leaving 75 mL of headspace, and 129 mL of headspace, respectively. Flasks were spiked with stock EDB, prepared in autoclaved distilled water, and incubated inverted  $23 \pm 1$  degrees C. The large headspace in the flasks served as a reservoir for oxygen and was monitored in a separate identical flask preparation. Actual oxygen levels in the experimental flasks were not measured. S1 flasks remained aerobic throughout the longest incubations (99 days), but oxygen in S2 flasks dropped by 90% after 7 weeks. Oxygen in S2 flasks was replenished by a flow of 1 headspace volume of air through a needle; displaced EDB was measured by sampling before and after this operation. Sulfate and nitrate levels persisted throughout the study period, evidence that aerobic conditions were maintained. Sulfate and nitrate disappeared from other flasks deliberately made anaerobic by sparging. Periodically, after brief shaking, subsamples were withdrawn.

S2 was suspended in distilled water spiked with radiolabeled EDB (60 ug EDB/liter) and carbon product fractionation performed. The headspace was

sufficient to keep the culture aerated for the 13 days of the experiment.

A replicate vial was taken periodically, and samples were assayed for radioactivity and EDB concentration. A purge train described by Bouwer and McCarty (1) comprised of a Tenax column trap, a pair of carbon dioxide traps, a heated stainless steel coil, a second pair of carbon dioxide traps. The purged culture was extracted with hexane and then filtered. The solids were combusted in a biological oxidizer and the radiolabeled carbon dioxide was collected in trap/scintillation fluid. Recovery with this instrument and radiolabeled (C14) sucrose standards was  $96.4 \pm 0.8$ . Production of radiolabeled carbon dioxide by soil microbes was confirmed.

The method of Barnhart and Vestal (2) for measuring the extent of acetate incorporation into microbial lipids was used to assess EDB inhibition of microbial activity. S1 or S2 was spiked with EDB in distilled water and incubated at 25 degrees C for either 3 or 12 hours. The initial EDB concentrations were 0, 0.1, 1, 10, 50, 100, and 1,000 ppm.

Citations used in Text:

1. Bouwer, E. J. and P. L. McCarty, 1983, Transformations of halogenated organic compounds under denitrification conditions. Appl. Environ. Microbiol. 45:1295-1299.
2. Barnhart, C. L. H., and J. R. Vestal. 1983. Effects of environmental toxicants on metabolic activity of natural microbial communities. Appl. Environ. Microbiol. 46:970-977.

#### Result

: RESULTS: Aerobic degradation: EDB was almost completely degraded within 1 week in both S1 and S2 at initial concentrations of 6 to 8 mg/Liter. At termination of this study, the flasks were charged with hexane to extract EDB from the entire contents, including the headspace. GC analysis for EDB revealed it was near or below the detection limit of 0.02 mg/L, representing at least 99% removal.

The results of soil incubations at 15 to 18 ppm demonstrated that soil components can catalyze EDB hydrolysis or chemically react with EDB.

Carbon products were determined by incubating diluted S2 suspensions with radiolabeled EDB. Nearly all of the recovered C14 in active cultures was associated with unreacted EDB, carbon dioxide, or unextractable forms in filterable solids. The C14 as carbon dioxide and solids increased with increasing EDB removal. Recovered EDB accounted for virtually all of the C14 trapped by Tenax and extracted from the culture by hexane and ether.

The C14 bound to solids represents carbon metabolized into cell materials and paralleled the extent of radiolabeled carbon dioxide evolution. A small fraction of the bound C14 resulted from abiotic processes. A small percentage of initial C14 which non-extracted appears to include products of both biotic and abiotic reactions, consisting of metabolic intermediates of EDB and water soluble EDB hydrolysis products such as 2-bromoethanol and ethylene glycol.

Soil aerobes or aerobic consortia may use EDB as a source of carbon and energy. Approximately equal amounts of EDB are converted to carbon dioxide and cellular carbon. Degradation at ppb levels was rapid and complete, whilst degradation at ppm levels was slow. EDB loss and bromide ion appearance and irreversible incorporation of C14 into soil particles of sterile controls indicate soil-mediated chemical mechanisms of EDB destruction may also occur.

#### Reliability

: STATISTICAL METHODS: Standard derivations were calculated.  
(2) valid with restrictions



### 3. Environmental Fate and Pathways

Id 106-93-4  
Date 28.12.2001

19.12.2001

(3)

Type  
Inoculum  
Method

: aerobic

:

: **STUDY OBJECTIVES:** The studies reported in the patent issuance document were used as supporting evidence for the effectiveness of the patented method for removal of residues of low molecular halogenated aliphatic hydrocarbons from ground and surface water under aerobic conditions.

**RECOGNIZED METHOD:** The authors indicated that this was a unique process for remediation of contaminated ground or surface water. The reference OECD guideline is OECD 307.

**GLP:** Pre-GLP.

**TEST MATERIAL:** Numerous low molecular weights halogenated aliphatic hydrocarbons that included ethylene dibromide (EDB).

**Purity:** Purity and the source suppliers of the starting materials were not given in the report

**CONTROLS:** As a comparison of the biological effectiveness of the microorganisms in degrading the halogenated aliphatic hydrocarbons, the test column was sterilized using 2 grams of sodium azide in water with a concentration of the halogenated aliphatic hydrocarbon being treated.

**CONCENTRATIONS OF TEST SUBSTANCES:** The report did not give the specific concentrations of the starting halogenated aliphatic hydrocarbons

**EXPOSURE PERIOD:** This was a continuous process. The results (i.e. rate of removal) were given per hour.

**EQUIPMENT:** No specifications of the types equipment used in the study were given.

**TEST PROCEDURE:** In the method, the use of natural occurring microbes (having the ability to use a class of enzymes called monooxygenases) to degrade low molecular halogenated aliphatic hydrocarbons. Sandy soil was packed into a glass column to a depth of 150 cm. A stream of air containing 0.6 percent natural gas by volume was passed over the head of the column. Three weeks were allowed for acclimation, after which the soil received water, which contained the specific halogenated aliphatic hydrocarbon. The concentrations of the halogenated aliphatic hydrocarbon in the column effluent were monitored for two weeks. The water containing the halogenated aliphatic hydrocarbon was applied at the rate of 21 cm per day. The elution volume of the column was 41 cm of water. Water entering and leaving the column pass through 16 mL screw cap test tubes that were sealed with a Telfon faced septum. As appropriate, the tubes were removed for analysis of the halogenated aliphatic hydrocarbon according to the USEPA test method no. 601. The tubes were then left in place long enough for 15 to 25 flushings before the samples were taken. With the exception of the sampling method, the construction and operation of the column was the same as described by Wilson (1981).

The first study used trichloroethylene to demonstrate the effectiveness of the method.

To confirm that the increased removal represented biological activity, the column was poisoned with water that contained 224 mgrams of trichloroethylene and 2 gram per liter of sodium azide. The amount of trichloroethylene passing through the column in the effluent was measured.

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A second study was carried out using radiolabelled radiolabeled (C14) trichloroethylene to identify the biotransformation products formed. After a second column was acclimated to natural gas, it was then dosed with a solution of radiolabeled (C14) trichloroethylene. After 1.6 solution volumes of water had been applied,  $15.8 \pm 0.3$  percent of the applied radiolabel appeared in the column effluent. This label could not be purged from the solution by vigorous aeration when the pH was adjusted to 11. At least 97 percent of the label precipitated with barium hydroxide indicating the biotransformation product was mainly carbon dioxide (radiolabeled C14).

A third study was run to illustrate the effectiveness of the method on twelve halogenated aliphatic hydrocarbons.

STATISTICAL METHODS: There is an implied statistical analysis of the results. However, the patent did not indicate how the data was handled. The percent removed was calculated as the material that passed through a living soil column divided by the material that passed through soil killed with 0.1% sodium azide times 100.

#### Result

: RESULTS: In the first analysis of the method only trichloroethylene (TCE) was evaluated and the removal of trichloroethylene was extensive, with less than 5 percent of the applied trichloroethylene passing through the soil column. After the sodium azide treatment, the amount of trichloroethylene passing through the soil increased significantly.

The second portion of the method analysis study showed the biotransformation products were mainly carbon dioxide.

The third portion of the method analysis compared the 12 aliphatic hydrocarbons, which included EDB. It demonstrated that EDB is effectively removed with 94% removed at a rate of 0.86 per hour., comparable to TCE wherein 95% was removed at a rate of 0.95 per hour.

Reliability  
19.12.2001

: (3) invalid

(45)

Type  
Inoculum  
Deg. Product  
Method  
Year  
GLP  
Test substance  
Method

: aerobic

:

:

:

:

: 1987

:

:

: STUDY OBJECTIVES: The objectives of this research were to examine the ability of a subsurface microbial community previously exposed to no known pollutants to degrade a wide variety of Xenobiotic compounds, determine rates of degradation, and identify patterns of degradation.

RECOGNIZED METHOD: No. Comparable to OECD guideline 307, Aerobic and Anaerobic Transformation in Soil

GLP: Not stated, although it is well established that this particular testing facility has complied with GLP regulations and principles since they were promulgated.

TEST SUBSTANCE: The radiolabeled substrates used included: m-[U-C14]cresol, [U-C14]ethylene dibromide, [U-C14]phenol, m-[U-C14]aminophenol, [U-C14]chlorobenzene, [U-C14]1,2,4-trichlorobenzene, p-[U-C14]chlorophenol, p-[U-C14]nitrophenol, [U-C14]aniline hydrochloride and [U-C14]barium carbonate. Purity: Not given.

OTHER MATERIALS: Aquifer solid samples were aseptically taken from an uncontaminated aquifer site, in unconsolidated material from the margin of

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the flood plain of a small river near. Uniform fine sand from the saturated zone of the profile was used in all experiments. This area of the profile, at a depth of 4.5 to 5.6 m below the surface, was under artesian head. There was approximately 0.22 mL of pore water per g of soil in this layer of the aquifer.

**CONTROLS:** Sodium azide was added as a metabolic inhibitor.

**CONCENTRATIONS OF TEST SUBSTANCES:** Two concentrations were used, approximately 10 to 50 and 500 to 1,000 ng/g of soil.

**EXPOSURE PERIOD:** Varied

**EQUIPMENT:** The amount of activity recovered (disintegrations per minute) was determined by using a Packard Tri-Carb 300D liquid scintillation counter.

**TEST PROCEDURE:** To evaluate the rates of mineralization by the microbial community, radiolabeled carbon dioxide evolution from a number of radiolabeled compounds was monitored over time. Because the microbes originated from aquifer solids, concentrations are presented on a solids dry weight basis. It should be recognized however, that all incubation vials contained approximately 1 g of aquifer solids and 24 mL of sterile distilled water; thus the microorganisms were in a more dilute suspension than the concentrations reflect.

Aquifer material was placed in a blender with aerated sterile distilled water, and a slurry was made. Sodium azide was added as a metabolic inhibitor. The radiolabeled isotope was added to all of the vials, and the remaining volume was filled with sterile distilled water. The headspace-free vials were inverted during incubations, carried out in the dark at 17 degrees C.

Samples were taken at specific time intervals of replicate samples and controls. The samples were then acidified with phosphoric acid to pH 2, and a center well containing 0.15 mL of 1 N KOH was placed in the vial headspace. The vials were placed on a rotary shaker at 80 rpm for approximately 18 hours, during which the radiolabeled carbon dioxide was trapped in the base. The amount of activity recovered (DPM) was determined by using a liquid scintillation counter. The efficiency of this method for trapping radiolabeled carbon dioxide was determined. An increase in the rates of mineralization (as a function of degradation) of radiolabeled substrates with exposure was used as an indication of adaptation.

Enumeration of specific degraders was based on the most-probable-number (MPN) technique (1) as modified by Somerville et al. (2). Two sets of MPNs were set up to assess changes in the numbers of degraders during adaptation; one set before adaptation, and the second set to assess the MPN after adaptation. After adaptation had been observed in the respiration experiment (day 35), radiolabeled p-nitrophenol (100 ng per vial) was added to all of the vials, which were allowed to incubate for an additional 26 days.

Replicate samples were scored positive if the radiolabeled carbon dioxide disintegrations per minute produced were greater than or equal to 3 times the control values for that dilution series. The MPN values were then calculated from these data by using the computer program of Clarke and Owens (3).

Citations used in Text:

1. Lehmicke, L. G., R. T. Williams, and R. L. Crawford. 1979. 14C-most-probable-number method for enumeration of active heterotrophic

### 3. Environmental Fate and Pathways

Id 106-93-4

Date 28.12.2001

microorganisms in natural waters. Appl. Environ. Microbiol. 38:644-649..  
2. Somerville, C. C., C. A. Monti, and J. C. Spanin. 1985. Modification of the <sup>14</sup>C-most-probable-number method for use with nonpolar and volatile substrates. Appl. Environ. Microbiol. 49:711-713.  
3. Clarke, K. R., and N. J. P. Owens. 1983. a simple and versatile micro-computer program for the determination of 'most probable number'. J. Microbiol. Methods 1:133-137.

#### Result

: RESULTS: The aquifer microbial community (solids) was capable of degrading a wide variety of xenobiotic compounds. The time frame of degradation varied from days for phenol, p-chlorophenol, and EDB, to weeks or months for m-cresol, m-aminophenol, aniline, and p-nitrophenol, and to years for the chlorinated benzenes.

EDB displayed a rapid rate of mineralization, initially, which leveled off such that a maximum percent respired was reached within weeks (@ 20-25%). Initial degradation rates were calculated by linear regression. The rate for EDB was 1.0% per day. A slightly greater percent of EDB was mineralized at the lower concentrations. The community was apparently already adapted to the utilization of EDB since no adaptation period was required before significant degradation occurred. No attempt was made, however, to identify the steps involved in the adaptation process.

Reliability  
19.12.2001

: (4) not assignable

(40)

Type  
Inoculum  
Deg. Product  
Method  
Year  
GLP  
Test substance  
Method

: anaerobic  
:  
:  
: other: Similar to OECD 307  
: 1986  
:  
: other TS: EDB; 99+% purity  
: STUDY OBJECTIVES: This study evaluates the degradation of EDB by hydrolysis in groundwater samples and by microbial activity in soil samples collected from north central and northwestern regions of Florida. Laboratory experiments were performed with natural samples in order to make qualified estimates of the persistence of EDB in the subsurface environment and to identify the products of degradation. The potential for biotransformation of the chemical by two different sludge preparations was also investigated.

RECOGNIZED METHOD: A specific guideline was not referenced. The experiment followed the scientific principles and procedures used in EPA/OPP and OECD accepted guidelines, such as OECD 307.

GLP: Not stated.

TEST SUBSTANCE: EDB was analytical grade with a purity of 99+% supplied by Aldrich Chemical, Milwaukee, Wisconsin. Radiolabeled EDB (<sup>14</sup>C, approximately 500 mCi/g) was obtained from Amersham, Arlington Heights, Illinois. The radiochemical purity was not given.

OTHER MATERIALS: Information on the source, purity and characteristics of all chemicals used in making the buffers were provided. Analytical grade chemicals were used.

#### CONTROLS:

Ground water Degradation Kinetics Study:

At least 2 samples in each incubation trial were fortified with radiolabeled EDB.

For the gas chromatography, standard curves of detector response over the range of 20 to 1500 pg/mL of EDB were constructed and used for quantitation using linear regression least squares analysis and were prepared daily.

For bromide analysis by absorbance, standard aqueous solutions of bromide were assayed to construct a standard curve over a range of 50 to at least 1000 ppb.

For scintillation counting, quenching was evaluated by comparing additions of the fortified waters or hexane extracts to additions of known amounts of radiolabeled toluene (C14). Efficiencies and precision of extraction of EDB from the waters were evaluated in a similar fashion.

#### Soil and Sludge Degradation Experiments:

An unfortified soil and three sterilized fortified soil replicates were analyzed at each sampling.

**CONCENTRATION OF TEST SUBSTANCES:** Groundwater samples and laboratory deionized water were fortified with EDB to concentrations of 10 to 100 ppb.

For the soil sludge experiments, fortification was carried out with EDB to a concentration of 400 ppb using an alcohol solution of radiolabeled EDB to give 4000-5000 dpm/mL as a tracer.

**EXPOSURE PERIOD:** Not specified for the groundwater degradation kinetics study. In the soil and sludge experiments, the soils and sludges were analyzed at 1, 5, 10, and 30 days then monthly thereafter for seven months.

**TEMPERATURE:** The hydrolysis portion of study was carried out at 40, 50, 60, 70, and  $80 \pm 0.5$  degrees C in the soil and in the sludge experiment the temperature was 25 degrees C.

#### TEST PROCEDURE:

##### Groundwater Degradation Kinetics Study:

Groundwater obtained from shallow wells in three north central and northwest Florida counties (Polk, Highlands, and Jackson) and laboratory-deionized water was fortified with EDB to concentration of 10 or 100 ppb. Samples were sterilized, microbial activity eliminated and incubated at 40, 50, 60, 70, and  $80 \pm 5$  degrees C. Aliquots were transferred to test tubes, hexane added, mixed for 5 minutes and sampled for gas chromatographic (GC) analysis.

At least 2 samples in each incubation trial were fortified with radiolabeled EDB and periodically sampled for total C14 activity to check the integrity of the system.

A series of  $5 \times 10^{-3}$  buffer systems were used to maintain the EDB fortified waters at pH values ranging from 4.0 to 9.0. The buffers included borax/succinic acid, phthalate/NaOH, borate/NaOH, borax/phosphoric acid, and carbonate/bicarbonate. Incubations were conducted at 62 degrees C.

Ethylene glycol concentrations in water were determined by GC analysis. The quantity of formaldehyde in the aqueous solution was also determined.

A Standard Methods Procedure (no. 405 C) was followed to measure the

bromide ion in aqueous solution. The bromide ion was oxidized by chloroamine T to bromine that brominates phenol red, and the absorbance of the reaction mixture was read at 590 nm.

#### Soil and Sludge Degradation Experiments:

Soils were sampled from three sites in Florida where water from wells had been contaminated with EDB. The EDB degrading ability of the indigenous microflora were examined. Samples from each site were collected at depths of 1 and 3 meters.

Two sludge sites were used to study the EDB degrading potential of a broad spectrum of microflora. One sludge sample contained a rich flora of facultative organisms. The second sludge sample was known to contain methanogenic flora.

The bottle-filling and incubation procedures were adapted from Bouwer and McCarty (1) and the medium was that of Alexander and Lurtigman (2). The sterilized medium (121 degrees C @ 15 psi, 20 min) was boiled and then flushed with N<sub>2</sub> while cooling and fortified with EDB to a concentration of 400 ppb. At each sampling, an unfortified soil along with three fortified and three sterilized fortified soil replicates were analyzed. At each sampling period, three 20 ml portions from each bottle were removed for carbon dioxide analysis.

STATISTICAL METHODS: Degradation rate constants were obtained by linear regression least squares analysis of plots of log % EDB remaining versus time. Pseudo-first order rate constants were used to generate Arrhenius plots (log rate constant versus 1/T degrees K) to estimate activation energies (E<sub>a</sub>) and to make extrapolated estimates of rate constants and half-life values at ambient temperature.

#### Result

#### : RESULTS:

#### Groundwater Degradation Kinetics Study:

All kinetic plots constructed from the data indicated that in the water tested, the disappearance of EDB from solution at all temperatures (40 to 70 degrees C) followed simple pseudo-first order kinetics. The rate constants observed in the different waters varied only slightly and indicate that neither acid nor base-catalyzed hydrolysis is favored within the pH range examined (pH 4-9). At pH 5 and 8 an increase in the rate constants of about 10% was observed, but because these portions of the plot were not consistent with a pH-dependent trend, i.e., a constant slope over a considerable pH range, these deviations may be attributed to specific contributions of the buffer type used.

The differences in the rates of degradation were evident in several groundwater samples. In contrast, the observed degradation rates in deionized water were identical. These results suggest that the constituents of groundwater may have an influence on the hydrolysis of EDB.

At elevated temperature (60, 72, and 80 degrees C) the decrease in radiolabel (C14) activity partitioned in the hexane phase after extraction paralleled the EDB decline determined by GC. Extractions of the aqueous phase did not result in detection of other brominated compounds. Nor were purgeable brominated compounds detected in the aqueous phase by GC/MS. The major hydrolysis products measured were EDB, ethylene glycol, and the bromide ion. During these incubations, these products accounted for 60 to 100% of the initial EDB.

Hydrolysis of EDB is a two-step process: first, the removal of bromide in the presence of water to form bromoethanol and second, the removal of

the second bromide ion to form ethylene glycol. The conversion of EDB to ethylene glycol and bromide ion was essentially complete after 7 days.

Prompted by a study that showed the EDB could be oxidized under anhydrous conditions to formaldehyde by the action of superoxide ion (6), water solutions were fortified with EDB and incubated at elevated temperatures for a period of time. Analysis found low consistent levels of formaldehyde, but only after all the EDB had been hydrolyzed. Studies conducted with natural and deionized water fortified to 10 ppm with ethylene glycol and incubated at 85 degrees C showed that the amounts of formaldehyde detected varied from about 350 ppb to 2 ppm after 40 days of incubation. These findings taken together indicate that hydrolysis of EDB is required as a first step for formaldehyde production and that EDB is not converted to formaldehyde by direct oxidation as was found in the superoxide study

Soil and Sludge Degradation Experiments: Soils and sludges did not show evidence of carbon dioxide production from EDB incubations. This result is consistent with negative results reported by Bouwer and McCarty (3,4) for carbon dioxide production.

In the methanogenic sludge, there was a decrease in dpm/mL found in the hexane extract with time, indicating the EDB was being degraded. The amount of dpm/mL in residual water, for both natural and sterile sludges indicates little dissolved carbon dioxide or other water-soluble degradation product(s) is being formed. This suggests the formation of a volatile product or products during incubation.

The results from the facultative sludge samples were similar to the methanogenic sludge. In 60 days, all the EDB was degraded and little radioactive material remained in the residual water after the triple hexane extraction. Similarly, the sludge produced the same gaseous products, of which only the ethylene was radioactive.

These results confirm the hypothesis of Bouwer and McCarty (3) that the water-insoluble volatile product derived from their EDB seeded culture incubations is ethylene. The results from these studies indicate a gradual decline of EDB prior to 40 days with a rapid decline thereafter.

The Florida soils tested were only weakly capable of degrading EDB under anaerobic conditions (e.g. 40% in labeled and non-labeled EDB after 7 months). No carbon dioxide was produced and there was no more radiolabel (C14) activity in the residual water after hexane extractions than for the sterile preparations. This indicates that the degradation product or products are volatile, such as ethylene. All other soils failed to degrade EDB under similar conditions over equally long incubation periods. This implies that either appropriate organism(s) are not present or the soils contained insufficient secondary carbon sources necessary to maintain co-metabolism.

#### Citations Used in Text:

1. Bouwer, E. J.; McCarty, P. L., Transformations of halogenated organic compounds under denitrification conditions. J. Appl. And Environ. Micro., 45(4)1286, (1983).
2. Alexander, M.; Lurtigman, B. K., Effect of chemical structure on microbial degradation of substituted benzenes, J. Agric. And Fd. Chem., 14,410 (1966).
3. Bouwer, E. J.; McCarty, P. L., Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions, Appl. And Environ Micro., 15(4)1286 (1983)
4. Bouwer, E. J.; McCarty, P. L., Ethylene Dibromide under methanogenic

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Id 106-93-4

Date 28.12.2001

**Reliability**  
19.12.2001

: conditions, Appl. And Environ Micro., in press (1985).  
: (2) valid with restrictions

(13)

**Type**  
**Inoculum**  
**Deg. Product**  
**Method**  
**Year**  
**GLP**  
**Test substance**  
**Method**

: anaerobic  
:  
:  
: other: Similar to OECD 307  
: 1986  
:  
: other TS: EDB; >97% purity  
: **STUDY OBJECTIVES:** The behavior of two groups of commonly occurring contaminants were examined in a synthetic microcosm constructed with authentic aquifer material that receives municipal landfill leachate and is known to support methanogenesis. The alkylbenzenes studied were benzene, toluene, ethylbenzene, and o-xylene, while the halogenated aliphatic hydrocarbons studies were 1,1-dichloroethylene, trans-1,2-dichloroethylene, cis-1,2-dichloroethylene, trichloroethylene and 1,2-dibromoethane.

**RECOGNIZED METHOD:** No. Most similar OECD test guideline is OECD 307, Aerobic and Anaerobic Transformation in Soil.

**GLP:** Not stated.

**TEST SUBSTANCE:** The chemicals used were high purity (>97%) benzene, toluene, ethylbenzene, o-xylene, radiolabeled (C14) toluene, 1,1-dichloroethylene, trans-1,2-dichloroethylene, cis-1,2-dichloroethylene, trichloroethylene, and 1,2-dibromoethane.

**OTHER MATERIALS:** Aquifer material for the anaerobic fate studies was obtained from sites adjacent to a landfill. Solid samples were taken by digging down to the region of methanogenesis and scooping up the aquifer material into sterilized 1-qt canning jars. The landfill was sited over highly permeable alluvium composed of silt, sand, clay, gravel, and dune sand. The depth of the alluvium varied from 1.7 to 1.22 m and lies over a 91-m layer of dense clay and chert gravel. The water table averaged from 0.6 to 1.5 m below the original land surface in areas adjacent to the river.

Liquid samples were taken by allowing the hole to fill with water and collecting the water in sterile glass containers. The aquifer water collected for the study was analyzed for metals, various parameters (pH, alkalinity, nitrate, etc.), and organic compounds.

**CONTROLS:** Sterile controls were autoclaved overnight at 121 degrees C prior to dosing.

**CONCENTRATIONS OF TEST SUBSTANCES:** Initial concentrations of EDB were 194 and 140 ug/L of pore water.

**EXPOSURE PERIOD:** 0, 3, 7, 16, and 40 weeks for the halogenated aliphatic hydrocarbons and 0, 6, 12, 20, 40, and 120 weeks for the alkylbenzenes.

**EQUIPMENT:** The analyses were conducted on a Finnigan Model 4000 GC/MS system interfaced to an INCOS data system in accordance with EPA Method 624. Both studies used a Hewlett-Packard 5880A gas chromatograph with flame ionization detection with nitrogen carrier gas at a 30 mL/min flow rate. The limit of detection for the alkylbenzenes and 1,2-dibromoethane was 0.1 mg/L; for the chlorinated hydrocarbons, the limit of detection was 1 mg/L.



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**TEST PROCEDURE:** All manipulations were performed in an anaerobic glovebox to ensure the maintenance of methanogenic conditions in the aquifer material, and all equipment in contact with the aquifer material was sterilized. The aquifer material was slurried by the addition of 15% by weight aquifer water and then poured into 160-mL serum bottles, resulting in approximately 100 g of aquifer material (wet weight) in each experimental unit. The dry weight of the solids averaged 67.5g. About 2 ml of dosing solution were added to the microcosms. The microcosms were stored upside down in the dark at 17 degrees C (the average groundwater temperature of the aquifer from which it was taken).

Two to four replicates of each treatment were analyzed at each sampling interval. Bottles were sampled by purging the volatile compounds onto a trap. Analysis was done by GC/FID.

The disappearance of halogenated aliphatic hydrocarbons from the methanogenic aquifer was measured at week 0, 3, 7, 16 and 40 after introduction of initial concentrations of these materials.

#### Result

: **RESULTS:** The disappearance of 1,2-dibromoethane was quite rapid; by 7 weeks of incubation, the concentration of 1,2-dibromoethane had been reduced to 27% of the control concentrations or less. At 16 weeks of incubation, the concentrations in the living samples were below the limit of detection. Chromatograms of samples at 3 and 6 weeks indicated the appearance of a new peak coinciding with the disappearance of 1,2-dibromoethane. GC/MS analyses at 6 weeks were unable to identify this peak. Transformation of 1,2-dibromoethane was also observed in the autoclaved controls of this study with the simultaneous appearance of a new peak in the chromatograms.

All four of the alkylbenzenes disappeared in the methanogenic aquifer material, but the disappearance was not rapid; long lag times were required before significant removal of all compounds was observed. Within the first 6 weeks of incubation, toluene concentrations were reduced to 13% of the original concentrations. Benzene, ethylbenzene, and o-xylene were not significantly degraded during the first 20 weeks of incubation. At 120 weeks of incubation, the concentrations of all four compounds were reduced to less than 1% of the original concentrations in the living samples. In contrast, there was no evidence of loss of benzene, toluene, ethylbenzene, or o-xylene in the autoclaved aquifer material at the 40 week interval. By 120 weeks of incubation the concentrations in the autoclaved samples for benzene, toluene, ethylbenzene, and o-xylene were 70%, 67%, 73%, and 66% of the original concentrations, respectively. Presumptive evidence of biological transformation of all four alkylbenzenes was based on extensive removal of material in living samples compared to autoclaved controls.

**Reliability**  
19.12.2001

: (2) valid with restrictions

(44)

**Deg. Product**  
**Method**  
**Year**  
**GLP**  
**Test substance**  
**Method**

:  
: other: OECD Guideline 307, Aerobic and Anaerobic Transformation in Soil  
: 1987  
:  
: other TS: Radiolabeled EDB  
: **STUDY OBJECTIVES:** To test whether EDB was available for transformation by naturally occurring microorganism.

**RECOGNIZED METHOD:** No. OECD Guideline 307, Aerobic and Anaerobic Transformation in Soil.

**GLP:** Not stated.

**TEST SUBSTANCE:** Radiolabeled EDB;Purity: Not given.

### 3. Environmental Fate and Pathways

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|                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                           | EXPOSURE PERIOD: 24 - 38 days.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Result                    | <p>TEST PROCEDURE: Soil that was contaminated with EDB (&lt;200 ppb) was suspended in water (1:1) and amended with radiolabeled EDB. At specific times, replicates were taken for methanol extraction to recover total EDB, that was then analyzed by GC and liquid scintillation counting.</p> <p>: RESULTS: Comparison of native field residues to radiolabeled EDB amended residues showed the amended residues were rapidly and nearly complete degraded. There was little or no degradation of the "native" field residue after 24 -38 days. The author notes that in other experiments radiolabeled EDB was converted to radiolabeled carbon dioxide (about 45%) and unextractable C14 associated with solids and taken to be cell material (about 55%).</p> <p>One of these soils was further incubated with radiolabeled EDB at 3 degrees C to reduce microbial degradation and allow greater time for radiolabeled EDB to penetrate particles. Some sets of replicates were warmed to 25 degrees C to monitor radiolabeled EDB degradation. 97% of the radiolabeled EDB was degradable.</p> <p>The author concludes from this that the native EDB in previously fumigated soils is inaccessible to microbes. Furthermore, EDB in this state does not exchange chemically with added radiolabeled EDB. Further evaluation of this native EDB demonstrated that it can be released following pulverization, but not via anionic and non-ionic surfactants, that had no effect upon its release. It was suggested that EDB from prior use had become entrapped in intraparticle micropore sites which is the reason for its persistence in topsoils.</p> |
| Reliability<br>19.12.2001 | : (3) invalid (1)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Deg. Product              | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Method                    | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Year                      | : 1990                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| GLP                       | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Test substance            | : other TS: EDB                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Method                    | : STUDY TYPE: Information Review                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                           | <p>RECOGNIZED METHOD: The paper addresses the following endpoints: OECD 103, Melting Point; OECD 104, Vapor Pressure; OECD 107/117, Octanol/Water partition coefficient; OECD 105, Water Solubility; and OECD 106, Adsorption/desorption to soil.</p> <p>GLP: Not stated.</p> <p>TEST SUBSTANCE: Ethylene dibromide</p> <p>OTHER MATERIALS: Not Applicable</p> <p>CONTROLS: Not Applicable</p> <p>CONCENTRATION OF TEST SUBSTANCES: Not Applicable</p> <p>EQUIPMENT: Not Applicable</p> <p>TEST PROCEDURE: Not Applicable</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Result                    | : RESULTS: This paper is a review of existing information taken from other scientific papers. There is no way to know the validity and soundness of the information in this paper.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

The authors summarize hydrolysis studies by several authors and note that

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at normal environmental temperatures, the half-life of EDB is at least 2 years, with ethylene glycol the major product. They also noted that EDB may react with naturally occurring sulfur nucleophiles (e.g. hydrogen sulfide and bisulfide ion) in ground water (anaerobic). The impact of microbial metabolism was also explored. A number of pure and mixed cultures from soil, sediment and aquifer microcosms can degrade EDB. This occurs in both oxic and anoxic conditions. It is dehalogenated to ethylene gas under methanogenic conditions. Concentration in the environment is a factor since EDB is toxic to bacteria, suggesting this activity is relevant when EDB is greater than a few tenths of a ppm. Such conditions are likely to exist after soil fumigation. Monitoring results from several States performed in the 1980's were also presented and summarized. However, comparisons of these results is difficult because of the variation in study design and statistical basis. Nonetheless the authors conclude that EDB is present in groundwater samples resulting principally from its use as a soil pesticide, and secondarily from spills or leaks of leaded gasoline when used as an additive.

#### Citation Used in the Text:

1. Call F., (1957a) Determination of the vapor pressure of ethylene dibromide. J. Sci. Food Agric. 8:81-85.
2. Call F., (1957b) The diffusion of ethylene dibromide. J. Sci. Food Agric. 8:86-89.
3. Call F., (1957c) The mechanism of sorption of ethylene dibromide on moist soils, J. Sci. Food Agric. 8:630-639.
4. Steinberg, S. M., Pignatello, J. J., Sawhney, B. L., (1987) Persistence of 1,2-dibromoethane in soils: entrapment in intraparticle micropores. Environ. Sci. Technol., 21: 1201-1208.
5. Stephen, H., Stephen, T., (1963), Solubilities of inorganic and organic compounds. Macmillan, New York, NY.
6. Timmermans, J., Martin, F., (1926) The work of the International Bureau of Physical-Chemical Standards. II. Study of twenty hydrocarbons and halogen derivatives, J. Chem. Phys., 23:747-787.

**Reliability** : (2) valid with restrictions (10)  
19.12.2001

**Deg. Product** :  
**Method** :  
**Year** : 1983  
**GLP** :  
**Test substance** :  
**Method** : STUDY OBJECTIVES: To study the transformation of several organic solvents at concentrations commonly found in surface and groundwater under anoxic conditions in the presence of denitrifying bacteria.

RECOGNIZED METHOD: Does not conform to any recognized method, but it is most comparable to OECD 302, Inherent biodegradability.

GLP: Pre-GLP

TEST SUBSTANCES: Reagent grade compounds were used: Naphthalene (NAPH) and 1,4-dichlorobenzene (1,4-DCB); 99+% 1,2- and 1,3-DCB, 1,2,4-trichlorobenzene (1,2,4-TCB) and ethylbenzene (EB); Chlorobenzene (CB), bromodichloromethane (BDCM) and 96% bromoform (BF); chloroform (CF) and carbon tetrachloride (CT); 1,1,1-trichloroethane (1,1,1-TCE) and 1,2-dibromoethane (1,2-DBE); Dibromochloromethane (DBCM).  
The following radiochemicals were used: 1,4-dichloro[U-C14]benzene (16.1 mCi/mmol), [C14]CT (22 mCi/mmol), and 1,2-bromo[U-C14]ethane (14.6

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mCi/mmol).

[Note: C14 refers to radiolabel]

**OTHER MATERIALS:** The general growth medium used contained the following (per Liter): 100mg of ethanol, 450 mg of NaNO<sub>3</sub>, 100 mg of K<sub>2</sub>HPO<sub>4</sub>, 20 mg of MgSO<sub>4</sub> × 7H<sub>2</sub>O, 5 mg of FeSO<sub>4</sub> × 7H<sub>2</sub>O, 2 mg of Calcium chloride, 0.2 mg of Manganese Chloride×4H<sub>2</sub>O, and 0.1 mg of NaMoO<sub>4</sub>×2H<sub>2</sub>O. An excess of nitrate was provided to maintain redox conditions between those for aerobic and methanogenic decomposition

**CONTROLS:** Sterile controls were used

#### CONCENTRATION OF TEST SUBSTANCES:

Batch 1 was composed of EB, NAPH, CB, 1,2-DCB, 1,3-DCB, 1,4-DCB, AND 1,2,4-TCB at an initial concentration between 40 and 114 mg/liter.

Batch 2 was composed of CF, CT, 1,1,1-TCE, BDCM, DBCM, BF, AND 1,2-DBE at nominal initial concentrations of 60 mg/liter. Carbon-14-labelled 1,2-DBE was also included (~3,000 dpm/mL)

The test substances were dissolved in ethanol at a concentration of 40 mg/mL of ethanol. The aqueous solution added to the bottles was prepared by diluting a stock solution of the aromatic compounds in ethanol with deionized water.

**EXPOSURE PERIOD:** 11 weeks at 25degrees C

**TEST PROCEDURE:** For Batch 1, sterile 160-mL serum bottles were purged with nitrogen gas and completely filled with deoxygenated medium with an anaerobic pipette (1). Except for sterile controls, the medium was seeded with 2 mL of primary sewage effluent per liter. A sample of the dilute aqueous solution containing a mixture of the aromatic compounds (batch 1) and 1,4-dichloro[U-C14]benzene tracer (~2 mCi/mL) was added directly to each bottle, and sealed. All bottles were incubated without agitation in the dark at 25 degrees C and were periodically assayed for the specific aromatic compounds by the closed-loop stripping technique, gas chromatography, and flame ionization detection (2).

Batch 2 experiment was conducted the same as Batch 1. Carbon-14-labeled 1,2-DBE was also included (~3,000 dpm/mL). Samples were periodically assayed for the halogenated compounds by the pentane extraction GC method of Henderson et al. (3).

A third series of sterile controls and bacterial cultures were prepared with CT and carbon-14 tracer, initially at 75 mg/liter, to study transformation products. An aqueous solution containing CT (1.6 mg/mL) and carbon-14 tracer (~2 mCi/mL) was added (7.5 mL) directly to each bottle before sealing. After several weeks of incubation at 25 degrees C, the carbon-14 labeled transformation products were characterized for methanogenic batch cultures (4).

Carbon-14 activities in the three batch experiments were determined by liquid scintillation counting (5).

#### Citations used in Text:

1. Owen, W. R., D. C. Stuckey, J. B. Healy, Jr., L. V. Young, and P. L. McCarty, 1979, Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res.*, 13:485-492.
2. Grob, K., and F. Zurcher. 1975, Stripping of trace organic substances from water-equipment and procedures. *J. Chromatogr.*, 117:285-299.
3. Henderson, J. E., g. R. Peyton, and W. H. Glaze, 1976. A convenient

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liquid-liquid extraction method for the determination of halomethanes in water at the parts-per-billion level, p. 105-112. In L. H. Keith (ed.), Identification and analysis of organic pollutants in water, Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan.

4. Bouwer, E. J., and P. L. McCarty, 1983. Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol, 45:1286-1294.

5. Bell, G. G., and F. N. Hayes, 1958. Liquid scintillation counting. Pergamon Press, Inc., New York, New York.

**Result** : RESULTS: The results did not demonstrate that ethylene dibromide was transformed under anoxic conditions when nitrate is present as the electron acceptor, although some other halogenated aliphatic compounds may be transformed under anoxic conditions when nitrate is present. CT, BDCM, DBCM, and BF were the only compounds studied that were transformed in the presence of denitrifying bacteria after 8 weeks of incubation. Chlorinated benzenes, EB, NAPH, CF, 1,1,1-TCE, and 1,2-DBE were not significantly transformed in the batch cultures. The production of carbon dioxide from the decomposition of CT and the partial incorporation of carbon into cells (particulates) indicated removal by biotransformation, although how this was possible remains unexplained.

**Reliability** : (2) valid with restrictions  
19.12.2001 (38)

**Deg. Product** :  
**Method** :  
**Year** : 1987  
**GLP** :  
**Test substance** : other TS: Radiolabeled (C14) EDB (98% purity, 23 mCi/mmol) and EDB 99% pure.

**Method** : STUDY OBJECTIVES: Examine the persistence of EDB from surface soils as a source of groundwater contamination. This report describes the desorption and bioavailability of residual EDB from Fumigated soils (referred to a "native" EDB) compared with added radiolabeled (C14) EDB.

RECOGNIZED METHOD: No. Most closely related to OECD 307, Aerobic and Anaerobic Transformation in Soil

GLP: Pre-GLP.

TEST SUBSTANCE: Radiolabeled (C14) EDB (98% purity, 23 mCi/mmol) and EDB 99% pure.

OTHER MATERIALS:

Soils: Cheshire fine sandy loam was obtained from a plot that was fumigated once at about 70 kg/ha in July 1985 by injection of EDB at a depth of 15 cm and 25 cm apart in a grid. Samples of two other soils were collected from former tobacco farms where EDB was presumed to have been applied to these fields according to standard agricultural practices at the rate of 70 kg/ha (frequency and total quantity applied are unknown). EDB use one site was halted in 1983, whereas the last known application to other site occurred in 1973. Samples collected were from 0 to 20 cm below the surface.

CONCENTRATION OF TEST SUBSTANCES: Test substance varied depending on the experiment (see test procedure below).

TEST PROCEDURE: EDB residues in the soil were extracted with methanol and then transferred to hexane after dilution of the extract with water. The hexane layer was analyzed by gas chromatography (GC). EDB in some extracts was verified by GC/MS (1).

The amount of EDB volatilized from the soil was measured using dry nitrogen gas. EDB determined by GC. The soil was then extracted with methanol and analyzed for the remaining EDB.

The soil/water partition coefficients ( $K_p$ ) were determined, and the concentration of sorbed EDB was calculated from the difference between added and supernatant counts.  $K_p$  was determined from the slope of linear plots of sorbed vs aqueous EDB concentrations. The apparent  $K_p$  for native EDB was determined under the same conditions.

The octanol-water partition coefficient was also measured.

The release of EDB into aqueous solution was measured using the purge technique and radiolabeled EDB. Purging was performed for 10-minute periods, after which time gas flow was stopped, and the hexane traps were changed. An aliquot of the hexane was added to 10 mL of scintillation fluid, and amount of radioactive EDB removed was determined.

Batch methods were utilized to determine the amount of EDB released from soil into aqueous solution, and at different temperatures. Soil suspensions were prepared. Checks at 25 and 75 degrees C showed that gentle agitation had no effect on release over the time periods used. Desorption rate and volatilization losses were also measured.

Biodegradation experiments were conducted using radiolabeled EDB. Samples used as controls contained NaN<sub>3</sub> as biocide. Total EDB was determined by GC analysis of the hexane layer. Radiolabeled EDB was determined by scintillation counting. The difference between the total EDB and radiolabeled EDB represented the native EDB which was not degraded.

The remaining methanol-extracted soil from each time point was further treated to determine the amount of fixed C<sup>14</sup> or the amount of radiolabeled EDB converted into cellular materials. Two additional replicates at each time point were examined for amount of evolved radiolabeled carbon dioxide.

#### Result

: RESULTS: Native EDB becomes entrapped in such a manner that it does not dissipate or become degraded. It is also essentially non-volatile and vigorous conditions are needed to extract EDB from environmental samples. The time from last known fumigation to sampling ranged from 0.9 to 13 years for the 3 agricultural sites. The amount of EDB determined as 27 ng/g (at 13 yrs) to 130 ng/g (at 0.9 yrs). The results indicate that EDB is not readily mobilized or degraded in the field.

The native EDB is also essentially nonvolatile. Air-drying soil samples in a Buchner funnel under aspirator suction for 24 hours did not decrease EDB concentrations.

Release of native EDB into aqueous solution from soil suspensions by purging with nitrogen gas showed that EDB was removed much more quickly from fresh soil samples compared to native EDB soil samples. Purging for 100 minutes removed the freshly added EDB completely while less than 5% of the native EDB was removed. An examination of particle size of soil samples demonstrated little impact upon EDB release.

Not only is the native EDB released slowly, but also it is not readily available for degradation by soil microbes. The aerobic degradation of a freshly added spike of radiolabeled EDB compared to that of native EDB in two soil samples demonstrated essentially no degradation of the native EDB occurred in either soil.

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Mechanical breakup of the soil particles in a ball mill resulted in accelerated release of EDB. The amount of EDB released from soil during a 15-minute extraction with water increased with time of pulverization from <0.1% before pulverization to >30% after pulverization for 10 minutes.

Pulverization also accelerated release into the vapor phase; the release of EDB was 40% from a sample pulverized for 5 minutes, compared to 8% from an unpulverized sample in a stream of N<sub>2</sub>.

EDB in fumigated soils is extremely resistant to volatilization, release into aqueous solution, and degradation by indigenous soil microbes. Pulverization promoted release, both to the aqueous and the gaseous phases.

The results suggest that EDB is entrapped in soil micropores. The very slow diffusion from these sterically complex structures is the determining factor for the rate at which equilibrium is established between EDB entrapped in the micropores and the gaseous or aqueous phase. The authors conclude that the non-degraded portion that is sorbed into soil particles may be important because it can slowly leach out over years and result in groundwater with concentrations of 0.1 ppb or less.

**Reliability**

19.12.2001

: (2) valid with restrictions

(28)

**Deg. Product**

:

**Method**

:

**Year**

: 1988

**GLP**

:

**Test substance**

:

**Method**

: STUDY OBJECTIVES: Purification of a dehalogenase from bacteria strain GJ70, a gram-positive actinomycete-like organism (previously identified as *Acinetobacter*) isolated from activated sludge.

RECOGNIZED METHOD: No. Most comparable to OECD Guideline 302, Inherent Biodegradability.

GLP: Not applicable

TEST SUBSTANCE: The dehalogenase was isolated from strain GJ70 grown to a density of 1mg/ml; cultivating conditions were: pH 7, 30 degrees C and 70% oxygen. These cells were harvested in a continuous centrifuge. Aliphatic dehalogenase was purified from crude extract dehalogenase from *Xantobacter*. Molecular weight and amino acid sequence were determined for the native and purified enzyme.

CONTROLS: Not applicable

CONCENTRATIONS OF TEST SUBSTANCES: Not applicable

TEST PROCEDURE: Aliphatic dehalogenase, purified from crude extract dehalogenase from *Xantobacter*, was examined for enzyme activity using 1-bromopropane as substrate. One unit of enzyme defined as the activity that catalyzes the formation of 1 umol halide/minute. Dehalogenase activity was determined at 30 degrees C and pH 8.8 in suitable buffer and substrate. Michaelis-Menten constants were determined from halide production curves. Vmax values were calculated from Lineweaver-Burke plots, and Km values derived from the same plot. The activity of the purified enzyme was measured against a broad range of brominated, chlorinated and iodinated compounds.

**Result**

: RESULTS: EDB was rapidly hydrolyzed by the dehalogenase, with the highest rate, expressed as a percent of the rate of the standard, 1-bromopropane. The rate was 172%. This paper covers that purification of a dehalogenase isolated from strain GJ70. The relationship to EDB is the

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|                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                  | <p>fact that there are microbes in the environment that have the ability to remove halogens from alkanes, alcohols, and ethers. It was proposed as potential mechanism for enzyme-catalyzed hydrolytic dehalogenation, demonstrating that EDB in the environment may be subject to biodegradation by microorganisms.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Reliability</b><br>19.12.2001 | : (4) not assignable (30)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Deg. Product</b>              | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Method</b>                    | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Year</b>                      | : 1985                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>GLP</b>                       | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Test substance</b>            | : other TS: Reagent grade EDB                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Method</b>                    | : STUDY OBJECTIVES: This paper demonstrates that EDB is transformed at low concentrations (25 to 90 mg/L) under similar methanogenic batch and continuous-flow column conditions.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|                                  | <p>RECOGNIZED METHOD: No. Comparable to OECD guideline 307, Aerobic and Anaerobic Transformation in Soil</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                  | <p>GLP: Not stated.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                  | <p>TEST SUBSTANCE: Reagent grade EDB obtained from Matheson Chemical Co., Norwood, Ohio (purity not given). 1,2-dibromo[U-C14]ethane (14.6 mCi/mmol) obtained from Amersham Corp., Arlington Heights, Illinois.<br/>Purity: Not given.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|                                  | <p>CONTROLS: Sterile batch controls were run concurrently.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|                                  | <p>EXPOSURE PERIOD: For the batch cultures, the sterile controls were exposed for 0, 1, 2, 4, 14, and 17 weeks. The seeded culture batches were exposed for 2, 4, 14, and 17 weeks.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                                  | <p>EQUIPMENT: EDB was measured with a detection limit of 0.1 mg/Liter in water by using a pentane extraction, gas-chromatographic procedure with electron capture detection (1). Carbon-14 activity was determined by liquid scintillation counting with the channels ratio method for quench correction (2).</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|                                  | <p>TEST PROCEDURE: Methanogenic batch suspended-growth experiments and a continuous-flow biofilm column study were performed to demonstrate EDB transformation. Sterile serum bottles containing deoxygenated medium for cultivating methanogens (3) were inoculated with EDB and a methanogenic mixed culture. These bottles were incubated in the dark at 35 degrees C and were periodically assayed for EDB and transformation products. A continuous-flow methanogenic column study was conducted for about 1 year with the same mixed bacterial culture inoculum. EDB and several other halogenated aliphatic organic compounds were applied simultaneously to the methanogenic biofilm column at concentrations ranging between 10 and 30 mg/liter. Details of these methods have been discussed by Bouwer and McCarty (4).</p> |
| <b>Result</b>                    | : RESULTS: EDB was transformed to below the detection limit in methanogenic cultures within the first 2 weeks of incubation (Table 1). Characterization of the remaining radiotracer activity indicated the formation of a highly volatile, nonhalogenated fraction, substantially more volatile than EDB (such as bromoethanol).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|                                  | <p>Under sterile conditions EDB is transformed by an abiotic process; whereas, in the presence of active microorganisms it was more rapid indicating that a biological mechanism predominated. Mineralization of EDB to carbon dioxide was not observed. EDB appeared to be reduced to</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |



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a highly volatile hydrocarbon, with the liberation of inorganic bromide. This result agrees with that of Castro and Belser (5), who showed a nearly complete conversion of EDB to ethylene and bromide by reductive dehalogenation in soil-water cultures.

The authors suggest that transformation may be an important mechanism for the removal of EDB that is present at low concentrations in reducing environments, particularly methanogenesis. Biological and abiotic processes both play a role. The biological processes is the most significant.

#### Citations used in Text:

1. Henderson, J. El, G. R. Peyton, and W. H. Glaze. 1976. A convenient liquid-liquid extraction method for the determination of halomethanes in water at the parts-per-billion level, p. 105-112. In L. H. Keith (ed.), Identification and analysis of organic pollutants in water. Butterworth's, Stoneham, Massachusetts.
2. Bell, G. G., and F. N. Hayes. 1958. Liquid scintillation counting. Pergamon Press, Inc., Elmsford, New York.
3. Owen, W. F., D. C. Stuckey, J. B. Healy, Jr., L. Y. Young, and P. L. McCarty. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res., 13:485-492.
4. Bouwer, E. J., and P. L. McCarty. 1983. Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. Appl. Environ. Microbiol. 45:1286-1294.
5. Castro, C. E., and N. O. Belser. 1968. Biodehalogenation. Reductive dehalogenation of the biocides ethylene dibromide, 1,2-dibromon-3-chloropropane, and 2,3-dibromobutane in soil. Environ. Sci. Technol. 2:779-783.

**Reliability**  
19.12.2001

: (4) not assignable

(37)

**Deg. Product**  
**Method**  
**Year**  
**GLP**  
**Test substance**  
**Method**

:  
:  
: 1980  
:  
:  
: STUDY TYPE: Structure-activity relationship approach

TESTING FACILITY: Not applicable.

STUDY NUMBER: Not applicable.

SPONSOR: Not applicable.

STUDY OBJECTIVES: Share experiences of using structure-activity relationships approach under the Chemical Control Law of Japan.

RECOGNIZED METHOD: No.

GLP: Not applicable.

TEST SUBSTANCE: Not applicable.

CONTROLS: Not applicable.

EXPOSURE PERIOD: Not applicable.

TEST PROCEDURE: There was no test procedure. Rather this is an examination of the structure activity of various classes of chemicals as they relate to biodegradation.

**Result**

: RESULTS: There are no specific results for EDB.

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Reliability : (4) not assignable  
19.12.2001

(11)

#### 3.6 BOD5, COD OR BOD5/COD RATIO

#### 3.7 BIOACCUMULATION

#### 3.8 ADDITIONAL REMARKS

## 4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type :  
 Species : Lepomis macrochirus (Fish, fresh water)  
 Exposure period :  
 Unit :  
 Analytical monitoring :  
 Method :  
 Year : 1959  
 GLP :  
 Test substance : other TS: Ethylene dibromide (research sample supplied by Dow Chemical Co.)  
 Method : SPECIES: Bluegill fish (Lepomis macrochirus Rafinsque). Fish were fingerling in size, 3-5 inches (@7.5-12.5 cm). Size range of fish was keep to a minimum. 10 fish per aquaria (25 L). Fish were obtained from a fish hatchery on the test site.

TEST CONCENTRATIONS: The median lethal limit (TLm) was determined for each chemical tested. The TLm is the inverse of the LD50; such that it is the concentration at which 50% of the test species are able to survive for a specified time period. 4 replicate aquaria with 10 fish per aquaria were used to validate the TLm values.

CONTROLS: Water samples were obtained from local waters, the Quachita River and the Bayou DeSiard (both near Monroe, LA).

TEST PROCEDURES: Species were collected from the hatchery, assayed and culled to a specific size and age. 10 fish were placed in 25 L aquaria and aerated to saturation. The water temperature was maintained at 25 degrees C. Water quality was determined including pH, oxygen, carbon dioxide and total solid analyses performed routinely during the exposure period. Fish were acclimated for 24 hours. EDB was added to the water to the predetermined application rate. Fish were observed for 1 hour after compound administration, and then at 6 hr intervals thereafter. 4 replicate aquaria with 10 fish per aquaria were used to validate the TLm values, which were determined by graphic interpolation.

EXPOSURE PERIOD: 24 and 48 hours

Result : RESULTS: EDB produced differences in survival between the two water samples used. In the Quachita River sample the TLm was 18 ppm at 24 hours, and in the Bayou DeSiard sample the TLm was 25 ppm at 24 hours and 18 ppm at 48 hours.

Reliability : (2) valid with restrictions

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(19)

Type :  
 Species : Micropterus salmoides (Fish, fresh water)  
 Exposure period :  
 Unit :  
 Analytical monitoring :  
 Method : other: Predates OECD and other national and international guidelines. The closest international guideline is OECD No. 203.  
 Year : 1959  
 GLP :  
 Test substance : other TS: Ethylene dibromide (research sample supplied by Dow Chemical Co.)  
 Method : SPECIES: Largemouth bass (Micropterus salmoides Lacepede). Fish were fingerling in size, 3-5 inches (@7.5-12.5 cm). Size range of fish was keep to a minimum. 10 fish per aquaria (25 L). Fish were obtained from a fish hatchery on the test site.

**TEST CONCENTRATIONS:** The median lethal limit (TLm) was determined for each chemical tested. The TLm is the inverse of the LD50; such that it is the concentration at which 50% of the test species are able to survive for a specified time period. 4 replicate aquaria with 10 fish per aquaria were used to validate the TLm values.

**CONTROLS:** Water samples were obtained from local waters, the Quachita River and the Bayou DeSiard (both near Monroe, LA).

**TEST PROCEDURES:** Species were collected from the hatchery, assayed and culled to a specific size and age. 10 fish were placed in 25 L aquaria and aerated to saturation. The water temperature was maintained at 25 degrees C. Water quality was determined including pH, oxygen, carbon dioxide and total solid analyses performed routinely during the exposure period. Fish were acclimated for 24 hours. EDB was added to the water to the predetermined application rate. Fish were observed for 1 hour after compound administration, and then at 6 hr intervals thereafter. 4 replicate aquaria with 10 fish per aquaria were used to validate the TLm values, which were determined by graphic interpolation.

|                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Result</b>                    | : EXPOSURE PERIOD: 24 and 48 hours<br>: RESULTS: EDB produced differences in survival between the two water samples used. In the Quachita River sample the TLm was 15 ppm at 24 hours, and in the Bayou DeSiard sample the TLm was 25 ppm at 24 hours and 15 ppm at 48 hours.                                                                                                                                                                                                     |
| <b>Reliability</b><br>19.12.2001 | : (2) valid with restrictions (19)                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>Type</b>                      | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Species</b>                   | : other: Common snook ( <i>Centropomus undecimalis</i> ) and Sheepshead minnow ( <i>Cyprinodon variegatus</i> ).                                                                                                                                                                                                                                                                                                                                                                  |
| <b>Exposure period</b>           | : 48 hour(s)                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Unit</b>                      | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Analytical monitoring</b>     | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Method</b>                    | : other: The closest international guideline is OECD No. 203.                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Year</b>                      | : 1984                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>GLP</b>                       | :                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Test substance</b>            | : other TS: Ethylene dibromide (source and purity not stated)                                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Method</b>                    | : SPECIES: Common snook ( <i>Centropomus undecimalis</i> ) and Sheepshead minnow ( <i>Cyprinodon variegatus</i> ). Common snook were juvenile seined from the Sebastian River. Juvenile sheepshead minnows were trapped from an estuarine pond at the Center for Marine Biotechnology (part of the Indian River system in east central Florida). Mean weight of fish was: Snook (0.25 grams) and Minnows (0.61 grams). Organism history and water quality data were not provided. |

**TEST CONCENTRATIONS:** Concentrations used with the common snook were 0.04, 0.2, 1, 5 and 10 mg/l. Concentrations used with sheepshead minnow were 0.1, 1, 10, 25 and 50 mg/l.

**CONTROLS:** Seawater and acetone controls were run concurrently.

**TEST PROCEDURES:** Species were collected, assayed and placed in plastic lined buckets, each containing 8 liters of filtered, diluted natural seawater. Buckets contained 6 snook or 5 minnows. Temperature of the water was similar to ambient temperatures. EDB was added to the water in acetone (1ml/8 liters of water). Lighting was continuous for 48 hours, temperature maintained at 22.9-25.9 degrees C. Fish were observed hourly for the first 12 hours and at intervals of 2-10 hours thereafter. Death was observed when opercular ventilation ceased or fish failed to respond to gentle prodding.

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STATISTICAL METHODS: The LC50's were calculated after 48 hours by the straightline interpolation method (American Public Health Association, Standard Methods for Examination of Water and Wastewater, 14th ed. , Washington, D.C., 1976)

**Result** : EXPOSURE PERIOD: 48 hours  
: RESULTS: EDB produced interference with osmoregulation, displayed as swelling of the abdomen. The 48 hours LC50 in Snook was 6.2 mg/liter, and in Sheepshead Minnows it was 4.8 mg/liter.

**Reliability** : (3) invalid  
19.12.2001 (2)

### 4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

**Type** :  
**Species** : other: Adult and F1 progeny of Hydra oligactis  
**Exposure period** :  
**Unit** :  
**Analytical monitoring** :  
**Method** : other: Relevant to OECD Guideline 202, and EPA OPPTS 850.1010.  
**Year** : 1988  
**GLP** :  
**Test substance** : other TS: Ethylene dibromide (source and purity not stated)  
**Method** : SPECIES: Adult and F1 progeny of Hydra oligactis. Source and loading not stated.

TEST CONCENTRATIONS: There were two separate experiments. First was an examination in adults using concentrations of: 7.5, 18.5, 30, 41.25, 52.5, 63.75 and 75 mg/l. In the second experiment, adults were pre-exposed to 5 mg/l and then concentrations of EDB used were: 25, 50, 100, 150, 200, 250 and 300 mg/l.

CONTROLS: Acetone control was used since it was used to emulsify and mix EDB with water. Concentration was 1500 mg/l dissolved in APW in experiment 1, and 6000 mg/l in experiment 2..

TEST PROCEDURES: In the first experiment using adults only, there were 2 control groups and 7 test groups. The acute response (i.e. motility and mortality) was measured in adults every 24 hours for 72 hours. Each test group consisted of 2 separate trials with 25 animals per trial. In the second experiment, adults were pre-exposed to 5 mg/l for 14 days and then exposed to one of 7 concentrations for 72 hours. There were 17 animals per dose group.

STATISTICAL METHODS: Statistically significant differences were measured against the control response.

**Result** : EXPOSURE PERIOD: 48 and 72 hours  
: RESULTS (First Experiment): The LC50 (adults only) determined from the plot of this response was calculated to be 70 mg/l after 48 and 50 mg/l after 72 hours.

RESULTS (Second Experiment): The LC50 after 72 hours determined from the plot of this response was calculated to be increased to 250 mg/l following pre-exposure. The effects in the pre-exposed offspring, F1 progeny, were even less pronounced than in their parents. At 72 hours the LC50 was approximately 300 mg/l.

**Reliability** : (3) invalid  
28.12.2001 (26)

## 4. Ecotoxicity

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Type :  
Species : other: Octopus joubini, Octopus maya and Octopus bimaculoides.  
Exposure period :  
Unit :  
Analytical monitoring :  
Method :  
Year : 1989  
GLP :  
Test substance : other TS: Ethylene dibromide (source and purity not stated)  
Method : SPECIES: Three species of octopuses used were: Octopus joubini, Octopus maya and Octopus bimaculoides. Hatchlings were cultured from these species in the laboratory. Animals were 3-4 months old and weighed 1-3 grams.

TEST CONCENTRATIONS: EDB was mixed with distilled water and added to seawater to make concentrations of 25, 50, 75 and 100 mg/l. A constant volume of 2 liters was maintained.

CONTROLS: Controls received distilled water equivalent in volume to the max dose of EDB solution.

TEST PROCEDURES: The study used cephalopods, advanced aquatic invertebrates, to measure the adverse effects associated with contaminants on marine animals. The source, age and culture of the species used were known. 2-4 animals were added to a 2.5 liter glass bowl containing 2 liters of artificial seawater (same seawater used for Octopus culture). Solutions of EDB were added to each bowl. Animals were exposed to 1 hour or 72 hours, after which animals were removed and placed in new bowls containing fresh seawater. Observations were made on behavior and mortality, hourly for the first 6 hours, and 2-10 hours during the chronic 72 hour exposure period. Water quality, including calcium and magnesium, was monitored throughout the experiment.

STATISTICAL METHODS: Results were statistically analyzed using Friedman non-parametric analysis of repeated measures.

### Result

EXPOSURE PERIOD: 1 and 72 hours  
: RESULTS: Immediate initial response was an intense dark brown coloration, followed by escape behavior. There was also a subsequent loss of locomotor response. Results were detected within 30 minutes at 25 mg/l, and recovery observed within 6 hours. Higher concentrations produced these response were prolonged. The rate of recovery after 1 hour exposure was concentration dependent. During recovery the animals displayed a mottled appearance indicating increased control of individual chromatophore motor units. Recovery was complete in 24 hours.

Lethality was produced in 100% of the animals in 3 hr at 100 mg/l. Mortality was observed at 25 mg/l during the chronic exposure.

Reliability : (2) valid with restrictions  
28.12.2001

(6)

## 4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : other algae:  
Endpoint : other: photosynthesis  
Exposure period :  
Unit :  
Analytical monitoring :  
Method : SPECIES: Phytoplankton species obtained from seawater pumped from

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the North Edisto River. Species included: Chlorophyceae, Cyanophyceae and Bacillariophyceae. Taxonomic identities of all collected species were determined.

TEST CONCENTRATIONS: Concentrations used were: 0.5, 1 and 2 mg/l.

CONTROLS: Control aquaria were maintained with all experiments.

TEST PROCEDURES: Native species of phytoplankton were collected from water pumped from the North Edisto River. Water was turbid, with 23-37 mg/L dry weight of suspended solids. Water pH was 7.8 and water temperature was 23.5-25 degrees C. Salinity was 21-22 g/L. Test aquaria were 37 liters. Water was delivered from a central location at the rate of 40 L/hr., and the mean turnover rate was 1.08 times /hr. Approximate time for replacement of water was 4.5 hrs (99%). Solutions of EDB were metered into the aquaria at pre-determined concentrations. Samples were collected from aquaria after 24 hours. Photosynthesis was measured by the uptake of radiolabeled Carbon (C14) from sodium bicarbonate (0.04 microcuries/ml) after incubation for 4 hours at 20 degrees C. Following incubation, cells were collected and radioactivity measured with a liquid scintillation spectrometer.

STATISTICAL METHODS: Data evaluated for significance from controls by multiple t-tests.

### Result

EXPOSURE PERIOD: 48 hours  
: RESULTS: Study is an acceptable measure of the impact on photosynthesis in native caught phytoplankton species from local waters. EDB produced no statistically measurable effect on the radiolabelled Carbon (C14) uptake by the estuarine phytoplankton.

### Reliability 28.12.2001

: (2) valid with restrictions

(42)

## 4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

### 4.5.1 CHRONIC TOXICITY TO FISH

### 4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

### 4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

### 4.6.2 TOXICITY TO TERRESTRIAL PLANTS

|                 |   |                                                                                                                                                                                                                   |
|-----------------|---|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Species         | : |                                                                                                                                                                                                                   |
| Endpoint        | : | other: photosynthesis                                                                                                                                                                                             |
| Exposure period | : |                                                                                                                                                                                                                   |
| Unit            | : |                                                                                                                                                                                                                   |
| Method          | : |                                                                                                                                                                                                                   |
| Year            | : | 1980                                                                                                                                                                                                              |
| GLP             | : |                                                                                                                                                                                                                   |
| Test substance  | : | other TS: Ethylene dibromide; technical grade from Eastman Kodak Co.                                                                                                                                              |
| Method          | : | SPECIES: Phytoplankton species obtained from seawater pumped from the North Edisto River. Species included: Chlorophyceae, Cyanophyceae and Bacillariophyceae. Taxonomic identities of all collected species were |

determined.

TEST CONCENTRATIONS: Concentrations used were: 0.5, 1 and 2 mg/l.

CONTROLS: Control aquaria were maintained with all experiments.

TEST PROCEDURES: Native species of phytoplankton were collected from water pumped from the North Edisto River. Water was turbid, with 23-37 mg/L dry weight of suspended solids. Water pH was 7.8 and water temperature was 23.5-25 degrees C. Salinity was 21-22 g/L. Test aquaria were 37 liters. Water was delivered from a central location at the rate of 40 L/hr., and the mean turnover rate was 1.08 times /hr. Approximate time for replacement of water was 4.5 hrs (99%). Solutions of EDB were metered into the aquaria at pre-determined concentrations. Samples were collected from aquaria after 24 hours. Photosynthesis was measured by the uptake of radiolabeled Carbon (C14) from sodium bicarbonate (0.04 microcuries/ml) after incubation for 4 hours at 20 degrees C. Following incubation, cells were collected and radioactivity measured with a liquid scintillation spectrometer.

STATISTICAL METHODS: Data evaluated for significance from controls by multiple t-tests.

**Result**

EXPOSURE PERIOD: 48 hours

: RESULTS: Study is an acceptable measure of the impact on photosynthesis in native caught phytoplankton species from local waters. EDB produced no statistically measurable effect on the radiolabelled Carbon (C14) uptake by the estuarine phytoplankton.

**Reliability**  
20.12.2001

: (2) valid with restrictions

(42)

**4.6.3 TOXICITY TO OTHER NON-MAMM. TERRESTRIAL SPECIES**

**4.7 BIOLOGICAL EFFECTS MONITORING**

**4.8 BIOTRANSFORMATION AND KINETICS**

**4.9 ADDITIONAL REMARKS**



## 5. Toxicity

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### 5.1.1 ACUTE ORAL TOXICITY

Type : LD50  
Species : rat  
Strain :  
Sex : male/female  
Number of animals : 100  
Vehicle : other: olive oil  
Method : other: Pre-dates OECD and US EPA Test Guidelines.  
Year : 1956  
GLP : no data  
Test substance : other TS: Ethylene dibromide; purity 99%  
Method : SPECIES/SEX: Male and female albino rats, from Dow Chemical stock colonies.

DOSE LEVEL(S) and NUMBER OF DOSES: Oral doses were given via gastric intubation, suspended in corn oil. There were 6 doses. It followed the Litchfield-Wilcoxon simplified method, using 10-20 animals in each group; doses spaced in geometric progression based upon results achieved with the previous dose. Aliquots of a 10% solution of EDB in olive oil were emulsified in 2 ml of a 5-10% acacia solution.

NUMBER OF ANIMALS/DOSE: A total of 100 rats (mixed sex) were used.

STUDY METHOD: Groups of young adult albino rats were administered single oral doses via gastric intubation according to their individual body weight. All surviving animals were observed until they had recovered fully from any loss of weight and were gaining normally. This period was typically 2 weeks.

Result : MEASURED ENDPOINT/INDEX (i.e. LD50, symptoms): LD50 = 0.14 grams/kg (mixed sexes).

RESULTS/OBSERVATIONS: LD50 was calculated according to the method of Litchfield-Wilcoxon.

Reliability : (2) valid with restrictions  
28.12.2001

(7)

Type : LD50  
Species :  
Strain :  
Sex :  
Number of animals :  
Vehicle : other: olive oil  
Method : other: Pre-dates OECD and US EPA Test Guidelines.  
Year : 1952  
GLP : no data  
Test substance : other TS: Ethylene dibromide; purity 99%  
Method : SPECIES/SEX: Rat, guinea pigs and rabbits were mature adults from Dow Chemical stock colonies; mice were young adults from Carworth Farms. Sexes tested as follows: Rats (m/f), mice (f), rabbits (f) and guinea pigs (m/f).

DOSE LEVEL(S) and NUMBER OF DOSES: Oral doses were given via gastric intubation. The number of doses was not reported but indicated it followed the Litchfield-Wilcoxon simplified method, which generally employs groups of 6-10 animals in each group; doses were spaced in geometric progression based upon results achieved with the previous dose. Aliquots of a 10% solution of EDB in olive oil were emulsified in 2 ml of a 5-10% acacia solution for rats, rabbits and guinea pigs. Aliquots of 1%

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solution in olive oil were used for mice.

NUMBER OF ANIMALS/DOSE: A total of 60 male rats, 40 female rats, 20 female mice, 55 female rabbits and 40 guinea pigs (mixed sex) were used.

STUDY METHOD: Groups of white rats, rabbits, mice and guinea pigs were administered single oral doses via gastric intubation according to their individual body weight. All surviving animals were observed until they had recovered fully from any loss of weight and were gaining normally. This period was typically 2 weeks.

### Result

: MEASURED ENDPOINT/INDEX (i.e. LD50, symptoms): Lethality in the species tested were determined as follows: LD50s (rat/m) = 0.146 grams/kg; (rat/f) = 0.117 grams/kg; (mice/f) = 0.420 grams/kg; (rabbits/f) = 0.055 grams/kg; and (guinea pigs/mixed) = 0.110 grams/kg .

RESULTS/OBSERVATIONS: LD50s calculated according to the method of Litchfield-Wilcoxon, and are reported above. The rabbits appear to be the most sensitive and mice the least sensitive species tested.

Reliability  
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: (2) valid with restrictions

(9)

### 5.1.2 ACUTE INHALATION TOXICITY

Type : LC50  
Species : other: Rats, Guinea Pigs and Rabbits  
Strain :  
Sex : male/female  
Number of animals :  
Vehicle :  
Exposure time :  
Method : other: Pre-dates OECD and US EPA Test Guidelines.  
Year : 1952  
GLP : no data  
Test substance : other TS: Ethylene dibromide; purity 99%  
Method : SPECIES/SEX: Rats and guinea pigs and rabbits were mature adults from Dow Chemical stock colonies. Both sexes were tested.

DOSE LEVEL(S) and NUMBER OF DOSES: Rats: 8 dose levels: 100, 200, 400, 800, 1000, 3000, 5000 and 10000 ppm. These doses are equivalent to: 0.77, 1.54, 3.08, 6.16, 12.3, 23.1, 38.5 and 77 mg/l, respectively. Guinea pigs: 2 dose: 200 and 400 ppm. Two separate groups of control animals, "air-exposed" control and "unexposed" control, were used throughout the study. The upper dose levels of 5000 and 10,000 were nominal values, while the lower concentrations were determined analytically.

NUMBER OF ANIMALS/DOSE: The number of animals varied per dose and exposure period. For example, a single dose of 1000 ppm had 4 separate exposure durations with 15-30 animals per exposure period. The 10000 ppm dose had 5 exposure durations and 4-20 animals/exposure period. The 100 ppm dose had 3 exposure durations and 20 animals/exposure. The same pattern was true for both species.

STUDY METHOD: Groups of mature adult rats and guinea pigs were exposed to volatilized concentrations of test material over varying lengths of time. The liquid material was metered into a 154 liter glass exposure chamber at a constant rate. Air flow in the chamber was regulated and vapor concentrations were checked by combustion and determination of total halogen. Body weights were measured. All surviving animals were observed until they had recovered fully from any loss of weight and were

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### Result

gaining normally. This period was typically 2 weeks. Selected animals were killed at 16-24 hours, their lungs, liver, heart, spleen, testes and kidneys weighed, and examined grossly and histologically.

: MEASURED ENDPOINT/INDEX (i.e. LC50, symptoms): The lethality at various doses and exposures were plotted and the LC99.99, LC50, and LC0.01 determined for each duration and dose. Mortality determined by the method of Litchfield and Wilcoxon (1949).

RESULTS/OBSERVATIONS: LC50s were calculated according to the method of Litchfield-Wilcoxon, and plotted for each duration and dose. For example, from the graph provided, an LC50 of 700 ppm can be estimated from a 1 hr exposure, 300 ppm for 3 hrs and 200 ppm for 10 hours of exposure. EDB had a slight anesthetic action but depression of the CNS occurred only at the higher doses. At high doses, deaths generally occurred within 24 hours from respiratory or cardiac failure; while at lower concentrations, deaths were delayed sometimes for 12 days resulting from pneumonia. Animals lost weight, coats appeared rough, they were irritable, discharged blood-tinged fluid from the nose and died. Examination of rats necropsied showed increased weight of lungs, livers and kidneys with numerous histologic changes. These consisted of congestion, edema, hemorrhage, and inflammation in the lungs. In the kidneys, slight interstitial congestion, edema and slight cloudy swelling of the tubular epithelium were observed. The livers displayed cloudy swelling, centrilobular fatty degeneration and necrosis.

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20.12.2001

: (2) valid with restrictions

(9)

### 5.1.3 ACUTE DERMAL TOXICITY

Type : LD50  
Species : rabbit  
Strain :  
Sex : no data  
Number of animals : 30  
Vehicle :  
Method : other: Pre-dates OECD and US EPA Test Guidelines.  
Year : 1952  
GLP : no data  
Test substance : other TS: Ethylene dibromide; purity 99%  
Method : SPECIES/SEX: White rabbits; sex not specified.

DOSE LEVEL(s) and NUMBER OF DOSES: 4 doses: 0.21, 0.30, 0.65 and 1.1 grams/kg

NUMBER OF ANIMALS/DOSE: There were 15 rabbits in the low dose (0.21 grams/kg) and 5 rabbits in each of the remaining 3 dose levels.

STUDY METHOD: The method followed that of Draize, Woodard and Calvery (1944) except the impervious sleeves were covered with heavy cloth bandages. Animals were not restrained. The occluded exposures lasted 24 hours after which the bandages were removed and exposed areas were cleansed with soap and water. Animals that survived were observed for 14 days.

### Result

: MEASURED ENDPOINT/INDEX (i.e. LD50, PII): An LD50 was not calculated. Deaths occurred as follows: 0.21 gm/kg (1/15), 0.30 gm/kg (2/5), 0.65 gm/kg (4/5) and 1.1 gm/kg (5/5).

RESULTS/OBSERVATIONS: EDB produced moderate to severe erythema, edema and necrosis. These usually healed with scarification. There was marked CNS depression and hypothermia at high doses. All

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deaths that occurred were experienced within 4 days of exposure.  
: (2) valid with restrictions

(9)

### 5.1.4 ACUTE TOXICITY, OTHER ROUTES

### 5.2.1 SKIN IRRITATION

### 5.2.2 EYE IRRITATION

Species : rabbit  
Concentration :  
Dose :  
Exposure Time :  
Comment :  
Number of animals :  
Result :  
EC classification :  
Method : other: Draize, Woodard and Calvery (1944).  
Year : 1952  
GLP : no data  
Test substance : other TS: Ethylene dibromide; purity 99%  
Method : SPECIES/SEX: White rabbits; sex not specified.

DOSE LEVEL(s) and NUMBER OF DOSES: 0.1 ml

NUMBER OF ANIMALS/DOSE: The number of rabbits was not specified.

STUDY METHOD: The method followed that of Draize, Woodard and Calvery (1944). All animal eyes were initially examined with fluorescein. 4 hours later the test material was introduced into one eye of each rabbit. After 30 seconds of contact the eye was flushed for about 3 minutes to remove the test material. Eyes were examined at 2, 24 and 48 hours, or until eye irritation effects had subsided.

Result : MEASURED ENDPOINT/INDEX (i.e. LD50, PII): Contact irritation effects on the eye.

RESULTS/OBSERVATIONS: EDB produced conjunctival irritation that cleared in 48 hours. A very slight corneal necrosis was observed, but healing was prompt and complete. A 10% solution in propylene glycol produced much more severe response with corneal injury lasting 12 days, but no adverse effects on the iris or lens.

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: (3) invalid

(9)

### 5.3 SENSITIZATION

### 5.4 REPEATED DOSE TOXICITY

Species : rat  
Sex : male/female  
Strain : other: Fischer 344 (CDF)  
Route of admin. : inhalation  
Exposure period : 13 weeks

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|                               |                                                                                                        |
|-------------------------------|--------------------------------------------------------------------------------------------------------|
| <b>Frequency of treatment</b> | : administered 6 hours per day, 5 days/week                                                            |
| <b>Post obs. period</b>       | : 88-89 days                                                                                           |
| <b>Doses</b>                  | : Dose levels of 0, 3, 10 and 40 ppm                                                                   |
| <b>Control group</b>          | :                                                                                                      |
| <b>NOAEL</b>                  | : = 3 ppm                                                                                              |
| <b>Method</b>                 | : other: Predates the OECD and US EPA Test guidelines.                                                 |
| <b>Year</b>                   | : 1980                                                                                                 |
| <b>GLP</b>                    | : no data                                                                                              |
| <b>Test substance</b>         | : other TS: Ethylene dibromide; 99.6% pure.                                                            |
| <b>Method</b>                 | : SPECIES/SEX: Fischer 344 (CDF) rats obtained from Charles River Labs; males and females 9 weeks old. |

ROUTE OF ADMINISTRATION: Inhalation.

DOSE LEVEL(s) and NUMBER OF DOSES: Dose levels of 0, 3, 10 and 40 ppm; administered 6 hours per day, 5 days/week for 13 weeks, for a total of 67 exposures. There were also 3 additional groups (10M/10F per group) for serial sacrifices: 1 week, 6 weeks and rats were held for a recovery period of 88-89 days and then necropsied to evaluate reversibility of effects.

NUMBER OF ANIMALS/DOSE: 10 male and 10 female rats per dose level. Animals were housed 2/cage during non-exposure, and 5 to 10/cage for females and males, respectively, during exposure periods.

STUDY METHOD: EDB was vaporized by metering the liquid at a calculated rate into a warmed vaporization flask (100 degrees C). The vapors were forced into the chamber with incoming and regulated. The nominal concentrations were calculated based upon the rate at which the liquid was dispensed and the total chamber airflow. Concentration was analyzed 3 times/day by GC with a flame ionization detector. Airflow was 300 ml/minute. The chamber was a 1 cubic meter stainless steel and glass Rochester type chamber. Chamber temperature was 21-28 degrees C and relative humidity was 43-46%. Body weights of each animal were obtained prior to start of study, twice a week for the first 2 weeks and then weekly throughout the study. Animals were observed for signs of toxicity.

HEMATOLOGY: Conducted on 7 males/dose prior to sacrifice at 6 weeks, and on 7/sex/dose at necropsy after 13 weeks of exposure, and after the recovery period. Parameters measured included: total erythrocyte count, total WBC, differential leukocyte count, hemoglobin, and PCV.

CLINICAL CHEMISTRY: Conducted on 7 males/dose prior to sacrifice at 6 weeks, and on 7/sex/dose at necropsy after 13 weeks of exposure, and after the recovery period. Parameters measured included: BUN, SGPT, SAP, glucose and bilirubin. Serum bromide levels were measured in rats exposed for 6 weeks.

URINALYSIS: Conducted on 7 males/dose prior to sacrifice at 6 weeks, and on 7/sex/dose at necropsy after 13 weeks of exposure, and after the recovery period. Parameters measured included: specific gravity, pH, glucose, ketones, bilirubin, urobilinogen, occult blood and protein.

ORGAN WEIGHTS: The following organs were weighed from all groups: liver, kidneys, testes, heart, thymus and brain. Absolute and relative organ weights were determined. Fasting body weights were used for organ to body weight ratios.

GROSS NECROPSY: Gross necropsy was performed on all animals on study, with special attention to the upper respiratory tract. Rats were fasted overnight prior to necropsy. The nasal passageways were perfused with

## Result

formalin fixative. A full compliment of tissues and organs were examined.

HISTOPATHOLOGY: Histopathological examinations were performed on a full compliment of tissues from all animals. It included the following reproductive organs: testes, ovaries, prostate, uterus, epididymides and accessory sex glands. In addition, transverse sections through the decalcified nasal cavity were prepared from 4 separate levels.

STATISTICAL ANALYSIS: Body weight, body weight gain, organ weight, urine specific gravity, hematology and clinical chemistry data were analyzed using ANOVA and Dunnett's test. The level of significance was  $p < 0.05$ .

GLP: Study pre-dates GLP; however, a Quality Assurance statement was provided.

: MEASURED ENDPOINT/INDEX (i.e. LD50, PII): A NOAEL of 3 ppm was determined. Study was preliminary to a long term 2-year carcinogenicity study that was planned.

RESULTS/OBSERVATIONS: NOTE: information which follows is for 13 week exposure period only. No difference between low dose and control animals was observed for the following: morality, moribundity, appearance, body weight, organ weight, hematology, biochemistry, urinalysis, gross necropsy or microscopic comparisons. There were no deaths in any group related to exposure.

Body weight was decreased in males 10 and 40 ppm, and in females at 40 ppm.

Hematology: The only effect was a decreased hematocrit and hemoglobin in females at 40 ppm.

Urinalysis: The only effect was a decrease in specific gravity in females at 40 ppm.

Clinical chemistry: There were no treatment related changes. The serum bromide levels were elevated above controls in a dose-related manner in all groups of males.

Organ-to-Body Weight Ratios: Relative liver weight increased in males at 40 ppm and in females at 10 and 40 ppm. Also, absolute liver weight increased in high dose females. Relative kidney weights increased in males only at 40 ppm.

Gross Necropsy: The only observation was pale livers in 50% of the high dose females.

Histopathology: Exposure related changes occurred primarily in the anterior portion of the nasal turbinates. At 40ppm there was slight diffuse or focal nonkeratinizing squamous metaplasia and hyperplasia of the respiratory epithelium in both sexes. At 10 ppm, there were slight degrees of isolated to multifocal hyperplasia of the respiratory epithelium, also in both sexes. Also, there was a slight increase in fatty livers in females at 40 ppm. There were no other significant histologic changes.

In the 88-89 day recovery group of rats, examination of the nasal turbinates demonstrated no discernible changes in comparison to controls, for males; while in females only 1/10 had a single focus of epithelial hyperplasia in the respiratory epithelium.

A NOAEL of 3 ppm was demonstrated.

## Reliability

: (1) valid without restriction

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(8)

|                               |   |                                                                                                                                   |
|-------------------------------|---|-----------------------------------------------------------------------------------------------------------------------------------|
| <b>Species</b>                | : | rat                                                                                                                               |
| <b>Sex</b>                    | : | male/female                                                                                                                       |
| <b>Strain</b>                 | : | Sprague-Dawley                                                                                                                    |
| <b>Route of admin.</b>        | : | inhalation                                                                                                                        |
| <b>Exposure period</b>        | : | 18 months                                                                                                                         |
| <b>Frequency of treatment</b> | : | 7 hrs/day 5 days/week                                                                                                             |
| <b>Post obs. period</b>       | : |                                                                                                                                   |
| <b>Doses</b>                  | : |                                                                                                                                   |
| <b>Control group</b>          | : |                                                                                                                                   |
| <b>Method</b>                 | : | other: Predates the OECD and US EPA Test guidelines; consistent with National Cancer Institute guidelines available at that time. |
| <b>Year</b>                   | : | 1979                                                                                                                              |
| <b>GLP</b>                    | : | no data                                                                                                                           |
| <b>Test substance</b>         | : | other TS: Ethylene dibromide; purity >99%.                                                                                        |
| <b>Method</b>                 | : | SPECIES/SEX: Weanling Sprague-Dawley CD rats; males weighing 131-134 grams, and females weighing 118-124 grams.                   |

ROUTE OF ADMINISTRATION: Test material administered via inhalation, 7 hrs/day 5 days/week for 18 months. Disulfiram, 96% purity, was mixed in the diet at a concentration of 0.05%.

DOSE LEVEL(s) and NUMBER OF DOSES: Test groups were as follows; Control, Control plus 0.05% disulfiram in the diet, EDB 20ppm, and EDB plus 0.05% disulfiram in the diet.

NUMBER OF ANIMALS/DOSE: 48 male and 48 female rats in each of the four groups.

STUDY METHOD: Each group of Sprague-Dawley CD rats were placed in a 4.5 cubic meters stainless steel, Rochester-type inhalation chamber and exposed to the test material vapors for 7 hrs/day, 5 days/week for 18 months. Rats were housed individually, and allowed free access to food and water except during exposure. The vapor was generated by bubbling nitrogen into a heated vessel containing EDB, maintained at 60 degrees C. EDB was mixed with incoming air and regulated at 736.3 l/minute. Airflow and EDB were both monitored. Concentration of EDB in the inhalation chamber during exposure was monitored via GC about 3 times per 7 hr exposure. Animals were rotated throughout the study so that by the 24th day they had been in every shelf and cage position. Chamber temperature and humidity were 70-80 degrees F and 35-55%, respectively. Animals observed twice daily throughout each exposure period for clinical signs of toxicity. Individual body weights were recorded weekly for the first 14 weeks, then monthly thereafter. Food consumption was recorded from the 4th month to the end of the study. Complete gross necropsy and histopathological examination were performed on all animals.

HEMATOLOGY: No measurements were taken, except as follows: anemic appearing rats in the 10th to 12th month were selected for measurement of hematocrit, hemoglobin, rbc and wbc. This included the following groups: EDB plus disulfiram and EDB without disulfiram.

CLINICAL CHEMISTRY: No measurements were taken.

URINALYSIS: No measurements were taken.

ORGAN WEIGHTS: The following organs were weighed from all groups at necropsy: spleen, liver, kidneys and heart. Absolute and relative organ weights were determined.

GROSS NECROPSY: Gross necropsy was performed on all animals on study.

HISTOPATHOLOGY: Histopathological examinations were performed on the following tissues from all groups: liver, heart, stomach, kidneys, pancreas, spleen, adrenals, thyroid, pituitary, urinary bladder, brain, skin, sternal bone marrow, lungs, mesenteric and tracheobronchial lymph nodes, salivary glands, zymbal glands, testes, prostate, mammary glands, ovaries, uterus, and abnormal appearing tissues. Nasal cavity was not examined owing the poor state of the preserved tissues.

STATISTICAL ANALYSIS: Differences between control and test values for body weight, food consumption and organ weights were compared by Tukey's omega procedure. Data on tumor incidence were analyzed by Fisher's exact probability test.

**Result** : MEASURED ENDPOINT/INDEX (i.e. LD50, PII): The study was conducted to determine whether ingestion of disulfiram during EDB exposures would alter the toxicity of EDB, since disulfiram inhibits acetaldehyde dehydrogenase and could inhibit the biotransformation of EDB.

RESULTS/OBSERVATIONS: Mortality was increased in the EDB and EDB plus disulfiram test groups. Mortality in the disulfiram plus EDB group at 12 months was 58% (M) and 83% (F). By the end of the 15th month all animals in this group had died or were terminated. Mortality in the EDB group at 18 months was 90% (M) and 77% (F). Body weight was also significantly decreased in both disulfiram groups, compared to controls. The effects in the EDB plus disulfiram group were more pronounced than the other groups. The EDB group gained weight comparable to controls, except for depressed body weights in the 15th and 18 months of exposure. Hematological measurements in the EDB and EDB/disulfiram groups demonstrated normal values for the EDB group, but EDB/disulfiram group had lower hematocrit, hemoglobin, and rbc counts. Meaningful organ weight comparisons could not be performed for the EDB groups because of the mortality that occurred in both groups. Two trends were, however, reported by the authors: increased absolute liver weight and decreased testes weight in the EDB/disulfiram group when compared to the EDB group only. All control organ weights were reportedly normal. Gross necropsy: Two significant findings were observed: (1) hemosiderosis in the spleens of EDB/disulfiram group (both sexes) and disulfiram alone (females), and (2) atrophy of the testes and prostate in the EDB/disulfiram group. Histopathology: Histopathological examination was essentially an elucidation of tumor incidences among groups. The control and control/disulfiram groups were comparable except for a marginal increase in mammary tumors for disulfiram females. EDB only: Males had increased tumors in spleen, adrenals and mesenchymal tissue; females had increased tumors in spleen, adrenals and mammary glands. EDB/disulfiram: tumor incidence was significantly increased in both sexes as follows: males - liver, spleen, mesenchymal tissue, kidney, adrenal, thyroid, brain and lung; females - liver, spleen, mesenchymal tissue, kidney, adrenal, thyroid, brain and mammary. Tumors were both benign and malignant.

**Reliability** : (2) valid with restrictions  
19.12.2001

(23)

**Species** : rat  
**Sex** : male/female  
**Strain** : Fischer 344  
**Route of admin.** : inhalation  
**Exposure period** : 88-103 weeks  
**Frequency of treatment** : 6 hrs/day 5 days/week  
**Post obs. period** :



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**Doses** : Test groups were as follows: 0, 10 and 40 ppm.  
**Control group** : yes  
**Method** : other: : OECD 451; consistent with National Cancer Institute guidelines available at that time.  
**Year** : 1982  
**GLP** : yes  
**Test substance** : other TS: Ethylene dibromide; purity >99%  
**Method** : ROUTE OF ADMINISTRATION: Test material administered via inhalation, 6 hrs/day 5 days/week for 88-103 weeks. Animals lived in the inhalation chamber throughout the study, except when being weighed or observed.

DOSE LEVEL(s) and NUMBER OF DOSES: Test groups were as follows: 0, 10 and 40 ppm.

NUMBER OF ANIMALS/DOSE: 50 male and 50 female rats in each group. Males were housed 3 per cage and females 4 per cage.

STUDY METHOD: Each group of Fisher 344 rats was placed in a 6 m<sup>3</sup> stainless steel inhalation chamber and exposed to the test material vapors for 6 hrs/day, 5 days/week for 88-104 weeks. Rats were allowed access to food and water 1 hour after exposure and until it was removed the next morning prior to exposure. EDB was mixed with incoming air and regulated at 1000 l/minute. Airflow and EDB were both monitored. Concentration of EDB in the inhalation chamber during exposure was monitored via GC 4 times per 6 hr exposure. Chamber temperature and humidity were 22 degrees C and 50%, respectively. Animals observed twice daily throughout the each exposure period for clinical signs of toxicity. Individual body weights were recorded monthly for the first 79 weeks, then twice monthly thereafter beginning at week 80. Complete gross necropsy and histopathological examination were performed on all animals.

HEMATOLOGY: No measurements were taken.

CLINICAL CHEMISTRY: No measurements were taken.

URINALYSIS: No measurements were taken.

ORGAN WEIGHTS: No organ weights were determined.

GROSS NECROPSY: Gross necropsy was performed on all animals on study.

HISTOPATHOLOGY: Histopathological examinations were performed on a full compliment of tissues, including but not limited to, the following tissues from all groups: adrenal, bile duct, brain, diaphragm, duodenum, epididymis, esophagus, eye, femur, heart, ileum, jejunum, kidney, large intestine, liver, lung, lymph nodes (cervical and mesenteric), ovary, pancreas, parathyroid, pituitary, preputial gland, prostate, salivary glands, seminal vesicle, skin, spleen, stomach, testes, thyroid, trachea, urinary bladder, uterus and vagina. Nasal cavity and sinuses were fixed whole in neutral buffered 10 formalin and/or Bouin's solution, decalcified and sectioned.

STATISTICAL ANALYSIS: Differences between control and test groups for effects on survival (Kaplan and Meier; Cox; Tarone's extensions of Cox for trend; and one-tailed P test), and incidence of neoplastic and non-neoplastic lesions (one-tailed Fisher exact test; Bonferroni test for inequality; Cochran-Armitage test for linear trend).

**Result** : MEASURED ENDPOINT/INDEX (i.e. LD50, PII): There was no NOAEL as the purpose of the study was to examine the carcinogenic potential to laboratory animals.

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**RESULTS/OBSERVATIONS:** Mean body weight of high dose males and females was lower than other groups. Mortality was increased in the high dose group. Survival in high dose decreased to 10% in males (wk 89) and 16% in females (wk 91), at which point all remaining animals in this group were sacrificed. Survival in control and low dose groups was comparable, with 70-78% survival to weeks 104-106.

**Non-neoplastic changes:**

High dose - increased incidence of hepatic centrilobular necrosis (M/F), toxic nephropathy (M/F), testicular degeneration, congestion and suppurative bronchopneumonia (M/F), suppurative inflammation and epithelial hyperplasia of the trachea (M/F), and inflammation and epithelial hyperplasia of the nasal cavity (M/F).

Low Dose - inflammation and epithelial hyperplasia of the nasal cavity (M/F), testicular degeneration, and retinal atrophy (F).

**Neoplastic changes:**

High dose - carcinoma and adenocarcinoma of the nasal cavity (M/F), hemangiosarcoma of the spleen (M/F), mesothelioma of the tunica vaginalis, bronchiolar carcinoma (F), mammary fibroadenoma (F), and invasive carcinoma in brain from nasal cavity (M/F).

Low dose - adenoma, adenocarcinoma and adenomatous polyp in nasal cavity (M/F), mesothelioma of the tunica vaginalis, and mammary fibroadenoma (F).

The test material was oncogenic in both sexes of rats at doses of 10 and 40 ppm.

**Reliability**  
18.12.2001

: (1) valid without restriction

(25)

**Species**  
**Sex**  
**Strain**  
**Route of admin.**  
**Exposure period**  
**Frequency of treatment**  
**Post obs. period**  
**Doses**  
**Control group**  
**Method**  
  
**Year**  
**GLP**  
**Test substance**  
**Method**

: mouse  
: male/female  
: B6C3F1  
: inhalation  
: 78-103 weeks  
: 6 hrs/day 5 days/week  
  
:  
: Test groups were as follows: 0, 10 and 40 ppm.  
: yes  
: other: OECD 451; consistent with National Cancer Institute guidelines available at that time.  
: 1982  
: yes  
: other TS: Ethylene dibromide; purity >99%  
: **ROUTE OF ADMINISTRATION:** Test material administered via inhalation, 6 hrs/day 5 days/week for 78-103 weeks. Animals lived in the inhalation chamber throughout the study, except when being weighed or observed.

**DOSE LEVEL(s) and NUMBER OF DOSES:** Test groups were as follows: 0, 10 and 40 ppm.

**NUMBER OF ANIMALS/DOSE:** 50 male and 50 female mice in each group. Mice were housed 4 per cage, by sex.

**STUDY METHOD:** Each group of B6C3F1 mice was placed in a 6 cubic meter stainless steel inhalation chamber and exposed to the test material vapors for 6 hrs/day, 5 days/week for 78-103 weeks. Mice were allowed access to food and water 1 hour after exposure and until it was removed the next morning prior to exposure. EDB was mixed with incoming air and

regulated at 1000 l/minute. Airflow and EDB were both monitored. Concentration of EDB in the inhalation chamber during exposure was monitored via GC 4 times per 6 hr exposure. Chamber temperature and humidity were 22 degrees C and 50%, respectively. Animals observed twice daily throughout each exposure period for clinical signs of toxicity. Individual body weights were recorded monthly for the first 79 weeks, then twice monthly thereafter beginning at week 80. Complete gross necropsy and histopathological examination were performed on all animals.

HEMATOLOGY: No measurements were taken.

CLINICAL CHEMISTRY: No measurements were taken.

URINALYSIS: No measurements were taken.

ORGAN WEIGHTS: No organ weights were determined.

GROSS NECROPSY: Gross necropsy was performed on all animals on study.

HISTOPATHOLOGY: Histopathological examinations were performed on a full compliment of tissues, including but limited to, the following tissues from all groups: adrenal, bile duct, brain, diaphragm, duodenum, epididymis, eye, femur, heart, ileum, jejunum, kidney, large intestine, liver, lung, lymph nodes (cervical and mesenteric), ovary, pancreas, parathyroid, penis, pituitary, preputial gland, prostate, salivary glands, seminal vesicle, skin, spleen, stomach, testes, thyroid, trachea, urinary bladder, uterus and vagina. Nasal cavity and sinuses were fixed whole in neutral buffered 10 formalin and/or Bouin's solution, decalcified and sectioned.

STATISTICAL ANALYSIS: Differences between control and test groups for effects on survival (Kaplan and Meier; Cox; Tarone's extensions of Cox for trend; and one-tailed P test), and incidence of neoplastic and non-neoplastic lesions (one-tailed Fisher exact test; Bonferroni test for inequality; Cochran-Armitage test for linear trend).

**Result**

: MEASURED ENDPOINT/INDEX (i.e. LD50, PII): There was no NOAEL as the purpose of the study was to examine the carcinogenic potential to laboratory animals.

RESULTS/OBSERVATIONS: Mean body weight of high dose males and females was lower than other groups. Survival was decreased in high dose females and low dose males. Survival in controls and low dose females and high dose male groups were comparable. The male portion of the study was run for 79 weeks and the females for 104 weeks.

**Non-neoplastic changes:**

High dose - increased incidence of serous inflammation of the nasal cavity (M/F), epithelial hyperplasia of the bronchus (M/F) and bronchiole (F), alveolar epithelial hyperplasia of the lung (F), and suppurative inflammation of the prostate.

Low Dose - increased incidence of serous inflammation of the nasal cavity (M/F), epithelial hyperplasia of the bronchus and bronchiole (F), and suppurative inflammation of the prostate.

**Neoplastic changes:**

High dose - carcinoma and adenocarcinoma of the nasal cavity (F), bronchiolar adenomas and carcinomas (M/F), subcutaneous fibrosarcoma (F), mammary adenocarcinoma (F), and hemangiosarcoma of the ovary.

Low dose - bronchiolar carcinomas (M/F), subcutaneous fibrosarcoma (F),

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and mammary adenocarcinoma (F).

The test material was oncogenic in both sexes of mice at doses of 10 and 40 ppm.

**Reliability** : (1) valid without restriction (25)  
20.12.2001

**Species** : other: Rats, Rabbits, Guinea Pigs and Monkeys  
**Sex** : male/female  
**Strain** :  
**Route of admin.** : inhalation  
**Exposure period** :  
**Frequency of treatment** :  
**Post obs. period** :  
**Doses** :  
**Control group** :  
**Method** : other: Pre-dates OECD and US EPA Test Guidelines.  
**Year** : 1952  
**GLP** : no data  
**Test substance** : other TS: Ethylene dibromide; purity 99%  
**Method** : SPECIES/SEX: Rats, guinea pigs and rabbits were mature adults from Dow Chemical stock colonies. Both sexes were tested. Age of monkeys was not given; both sexes were tested.

**DOSE LEVEL(S) and NUMBER OF DOSES:** The number of animals/dose varied with each protocol; e.g. some were exposed 7hr/day for 9 days, some for 91 days and some for 213 days. In all cases, however, two separate groups of control animals, "air-exposed" control and "unexposed" control, were used throughout the study.

**STUDY METHOD:** Groups of mature adult rats, guinea pigs, rabbits and monkeys were exposed to volatilized concentrations of test material over varying lengths of time. Animals were generally exposed 7 hrs/day 5 days/week. The liquid material was metered into a 154 liter glass exposure chamber at a constant rate. Air flow in the chamber was regulated and vapor concentrations were checked by combustion and determination of total halogen. Body weights were measured. Animals were weighed twice/week and observed for appearance and behavior. Growth curves and mortality records were kept for each group. Representative hematological parameters were determined in rats and monkeys. All moribund animals were killed and examined for evidence of any organ damage.

**HEMATOLOGY:** Blood that was collected was obtained at the time of autopsy and plasma-prothrombin-clotting time determined on animals exposed for 100 ppm.

**CLINICAL CHEMISTRY:** BUN determined from blood obtained at necropsy. Some liver sections obtained at necropsy were frozen in dry ice for subsequent total lipid analyses.

**URINALYSIS:** Not performed.

**ORGAN WEIGHTS:** The following organs were weighed: spleen, liver, kidneys, testes, heart, and lungs. Absolute and relative organ weights were determined.

**GROSS NECROPSY:** All animals were fasted overnight, except guinea pigs. Each animal was weighed and killed. Gross appearance was recorded and its lungs, liver, heart, spleen, testes and kidneys weighed, and examined grossly.

**Result**

HISTOPATHOLOGY: Tissues from these organs and the pancreas and adrenal glands were prepared and stained with hematoxylin and eosin. In some cases the liver and kidney were stained with Oil red O.

: MEASURED ENDPOINT/INDEX (i.e. LD50, PII): Studies conducted to determine vapor exposures that produce adverse effects at varying concentrations and duration of exposure. Levels below which such effects were observed or measured were estimated.

RESULTS/OBSERVATIONS: Results varied by species, concentration and duration, and are summarized below:

Rats (100 ppm for 7 days): 3/10 died after 1, 5 and 7 exposures; they lost weight and surviving animals were thin and unkempt; stomach contents were blood-tinged; lungs, liver and kidney weights were significantly increased; thickening of the alveolar walls and leucocytic infiltration in the lungs; cloudy swelling in the liver without fatty degeneration, and slight congestion and hemosiderosis of the spleen. BUN and other blood parameters were normal.

Rabbit (100 ppm for 3 days): 2/4 died after 2 exposures and another died after 3 exposures; necropsy revealed widespread central fatty degeneration of the liver, with necrosis. BUN and other blood parameters were normal.

Rat (50 ppm for 91 days): 10/20 males died due to pneumonia and upper respiratory tract infections; 4/20 females died. Animals displayed a reduced growth. In males there was increased liver, lung and kidney weights, while testes weights decreased. In females, liver and kidney weight increased, while spleen weights decreased. BUN and other blood parameters, and total lipid were normal. Pathology of the males showed pneumonic consolidation in the lungs. This was not evident in females. No effects observed in other organs.

Guinea Pig (50 ppm for 80 days): growth in both sexes was depressed, but no effect on mortality. Final body weights were significantly decreased. The absolute weights of lung, liver and kidneys were significantly increased. There was central fatty degeneration of the liver, and slight degenerative changes in the tubular epithelium of the kidney. All other parameters measured were normal.

Rabbit (50 ppm for 84 days): There was no evidence of any adverse effects in the parameters measured - growth, behavior, body weight, BUN or lipid. There were slight increases in liver and kidney weights.

Monkey (50 ppm for 70 days): Animals appeared ill and nervous throughout the study, and lost 5% of initial body weight. The only significant effect was slight liver weight increase with central fatty degeneration. Total lipid was slightly increased. All other parameters measured were normal.

Rat (25 ppm for 213 days): 10/20 males died due to pneumonia; 3/20 females died. Generally no adverse effects were observed, other than the mortality due to pneumonia.

Guinea Pig (25 ppm for 205 days): 4/8 males and 2/8 females died due to pneumonia. There were no other adverse effects observed.

Rabbit (25 ppm for 214 days): No adverse effects observed.

Monkey (25 ppm for 220 days): No adverse effects observed in any of the parameters measured.

**Reliability**  
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: (2) valid with restrictions

(9)

## 5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test  
System of testing : Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537 and TA1538, and Escheria coli WP2 her.  
Concentration : Doses used: 50, 100, 500, 1000 and 5000 ug/plate.  
Cycotoxic conc. :  
Metabolic activation : with and without  
Result : positive  
Method : OECD Guide-line 471 "Genetic Toxicology: Salmonella thyphimurium Reverse Mutation Assay"  
Year : 1983  
GLP : no data  
Test substance : other TS : 1,2-Dibromoethane obtained from Aldrich Chemical Company.  
Method : TEST ORGANISM USED: Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537 and TA1538, and Escheria coli WP2 her. Strains were cultured overnight in liquid nutrient broth and stored at -80 degrees C. Their genetic markers and response to positive controls and number of spontaneous revertants were checked.

TEST COMPOUND CONCENTRATIONS USED: 5 concentrations were evaluated with appropriate vehicle and positive controls. Doses used: 50, 100, 500, 1000 and 5000 ug/plate.

CONTROL MATERIALS: The positive control materials that were used were not identified in the published report. The negative control was Distilled water or DMSO.

ACTIVATION: Source and preparation of the S-9 fraction was not identified.

TEST PERFORMANCE: Salmonella typhimurium reversion assay as described by Ames et al. (1975).

PRELIMINARY CYTOTOXICITY ASSAY: The report did not indicate whether these had been done. Based upon the number of pesticides examined it is most likely this information was derived from the manufacturer or literature. The results were not expressed as the number of revertants per survivors, but as revertants per plate. Therefore, toxicity was not examined in this study.

PROTOCOL: Tests were carried out according to Ames et al. (1975). Each dose level of the compound was tested with and without the S9 mix with each strain of S. typhimurium. When E. coli was used, histidine and biotin in top agar were replaced by tryptophan at the same concentration. 0.1 ml of bacterial culture of each indicator strain were added to test tubes containing 2 ml of molten agar supplemented with biotin and a trace of histidine, 0.1 ml of the appropriate dilution/suspension of the test product and 0.5 ml of the S9 mix, if any. The ingredients were mixed and immediately poured onto the minimal agar plates with modified Vogel-Bonner E medium. After the top agar has set, the plates are incubated at 37 degrees C for 2 days. Plates were scored for number of revertants/plate.

Result : REPORT RESULTS: Test compound did induce a significant increase in the number of revertant colonies for Salmonella typhimurium strains TA1535, TA100, TA98 (weak) and in E. coli, with and without S9 activation, at doses up to and including 5000 ug/plate.

CONCLUSION: Test material was mutagenic in S. typhimurium TA 98,

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TA100 and TA1535 and E. coli WP2 her with and without S9 activation.

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**Type** : Mammalian cell gene mutation assay  
**System of testing** : TEST CELLS USED: Chinese hamster ovary (CHO) cells, subline CHO-K1-BH4.

Properly maintained: Yes, in Ham's F-12 medium with newborn calf serum (10%)

Periodically checked for mycoplasma: Not stated.

**Concentration** : 0-5 mM  
**Cytotoxic conc.** :  
**Metabolic activation** : with and without  
**Result** :  
**Method** : OECD Guide-line 476 "Genetic Toxicology: In vitro Mammalian Cell Gene Mutation Tests"

**Year** : 1982  
**GLP** : yes  
**Test substance** : other TS: 1,2-Dibromoethane obtained from Matheson, Coleman & Bell.  
**Method** : CONTROL MATERIALS:  
Ethyl Methanesulfonate (EMS)  
n-nitrosodimethylamine (DMN)  
Solvent Control: DMSO  
Metabolic Activation System:  
S9 from rats treated with Aroclor 1254, with three separate fractions (Mg-S9;Ca, Mg-S9;Ca-Mg-microsomes)

ACTIVATION: Aroclor 1254-induced rat liver S9 prepared from male Sprague-Dawley. Immediately prior to use, three variations of S9 were mixed as follows: Mg-S9: with 10 mM MgCl<sub>2</sub>, 4 mM NADP, 5 mM G-6-P, 30 mM KCl, 10 mM MgCl<sub>2</sub> and 50 mM NaP buffer, pH 8.0. Ca, Mg-S9: same as above with CaCl<sub>2</sub> included. Ca-Mg-Microsome: 1 ml of microsome preparation added to 9 ml of buffered salt solution above and 10mM CaCl<sub>2</sub> and 0.5 units of G-6-P.

TEST PERFORMANCE: CHO/HGPRT Mutation Assay: Cells were seeded in F12CM5 and Ham's F12 medium at a density 5 x 10E5 cells/25 square cm flask, and incubated at 37 degrees C in 5% CO<sub>2</sub> for 16 hrs. Cells to be treated in the presence of a metabolic activation system were washed once in saline G and the medium replaced with 4 ml of serum-free Ham's medium F12 and 1 ml of the appropriate activation system. Duplicate flasks were exposed to varying concentrations of test article for 5 hrs at 37 degrees C. Afterward, cells were washed with saline G, F12CM5 added, and incubated overnight. The next day an estimation of cytotoxicity by mutagen treatment was determined by measuring the cloning efficiency of 200 cells plated in triplicate. Cells were subcultured to determine phenotypic expression. At the end of the subculture period (1-8 days), mutation frequency expressed as the number of mutant colonies per 1x10E6 cells in 5 plates.

**Result** : REPORT RESULTS: Several experiments within the test, using different activation systems and differing concentrations of EDB demonstrated that EDB is more cytotoxic in the presence of Ca, Mg-S9 than without it; however, the mutagenic activity remained unchanged. Results supported the findings that metabolic activation does not increase the mutagenic activity of EDB, just the cytotoxicity. Further they concluded that the increase cytotoxicity was NADP dependent

CONCLUSION: It was not the intention to demonstrate whether or not EDB was mutagenic, but rather to examine its mutagenic potential under varying

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| <b>Reliability</b><br>20.12.2001                                                                                                                               | : activation systems and exploration of detoxifying mechanisms.<br>(3) invalid                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | (34) |
| <b>Type</b><br><b>System of testing</b><br><b>Concentration</b>                                                                                                | : Mammalian cell gene mutation assay<br>: Human lymphoblastoid cell lines, AHH-1 and TK-6.<br>: In AHH-1: 1, 2, 5, 10, 50 and 100 ug/ml.<br><br>In TK-6: 2, 5, 20, 40 and 60 ug/ml. All test chemical and negative controls were tested in triplicate, and all positive controls were tested in duplicate cultures.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |      |
| <b>Cycotoxic conc.</b><br><b>Metabolic activation</b><br><b>Result</b><br><b>Method</b><br><b>Year</b><br><b>GLP</b><br><b>Test substance</b><br><b>Method</b> | : Highest doses caused cytotoxicity<br>:<br>: positive<br>: other: Not stated; but consistent with OECD 476.<br>: 1985<br>: no data<br>: other TS: 1,2-Dibromoethane obtained from Aldrich Chemical Company.<br>: TEST ORGANISM USED: Human lymphoblastoid cell lines, AHH-1 and TK-6. Cell line AHH-1 contains mixed-function oxygenase activity; gene mutations are measured at the hgp <sup>r</sup> t locus (hypoxanthine guanine phosphoribosyl transferase) via resistance to 6-thioguanine. TK-6 cell line does not contain mixed-function oxygenase activity; they are heterozygous at the tk (thymidine kinase) locus and gene mutations are measured there via resistance to trifluorothymidine.<br><br>TEST COMPOUND CONCENTRATIONS USED: 5-6 concentrations, with the highest doses causing cytotoxicity. In AHH-1 the concentrations were: 1, 2, 5, 10, 50 and 100 ug/ml. In TK-6 the concentrations were: 2, 5, 20, 40 and 60 ug/ml.. All test chemical and negative controls were tested in triplicate, and all positive controls were tested in duplicate cultures.<br><br>CONTROL MATERIALS: The following control materials were employed.<br><br>Positive Control:<br>Non-Activation: 4-Nitroquinoline-N-oxide (70 ng/ml) used with TK-6<br>Benzo[a]pyrene (3.1 ug/ml) used with AHH-1<br><br>Negative control: DMSO<br><br>TEST PERFORMANCE: Background mutation frequency was reduced by treating TK6 and AHH-1 lymphoblasts for 48 and 72 hours, respectively, with CHAT (deoxycytidine, hypoxanthine, aminopterin and thymidine). Cells were centrifuged and resuspended in medium containing THC (CHAT without aminopterin). Cultures counted daily and exposed to test material 2-3 days after resuspension in THC. All cell manipulations after this point were conducted in medium containing penicillin and streptomycin. Cytotoxicity was determined by cell growth survival: treated response divided by negative control response to yield relative survival.<br><br>PROTOCOL:<br>TK-6: Each culture of 4-5 x 10E7 TK6 cells was exposed to test material for 20 hours (@1.5 cell generations). During phenotypic expression (3 days for tk locus), cell concentration was determined daily. Cultures were plated on the 3rd day after treatment in medium containing trifluorothymidine. It was heated at 56 degrees C for 1 hour and incubated for 12 days, then scored for the presence or absence of colonies.<br><br>AHH-1: Each culture of 4-5 x 10E7 AHH-1 cells was exposed to test material for 28 hours (@1.5 cell generations). During phenotypic expression (6 days for hgp <sup>r</sup> t locus), cell concentration was determined on |      |



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**Result** : days 0, 1, 3 and 5 after exposure. Cultures were plated on the 6th and 7th days after treatment in medium containing 6-thioguanine. It was incubated for 14 days, then scored for the presence or absence of colonies.  
: REPORT RESULTS: 1,2-dibromoethane was mutagenic in both human cell lines; at 5 ug/ml in AHH-1 cells, and at 20 ug/ml in Tk-6 cells. It demonstrated equal mutagenic potential in both cell lines. Although AHH-1 contains five times more glutathione S-transferase activity than TK-6 cells, there was no apparent increase in the mutagenic activity of 1,2-dibromoethane between the two cell lines.

**Reliability** : CONCLUSION: Test material was mutagenic in the in vitro mammalian cell gene mutation assay using human lymphoblasts.  
20.12.2001 : (2) valid with restrictions

(14)

**Type** : Unscheduled DNA synthesis  
**System of testing** : CDF (F344)/CrIBr male rats  
**Concentration** : 10, 50 and 100 uM in DMSO (1%) in vitro  
**Cycotoxic conc.** :  
**Metabolic activation** :  
**Result** :  
**Method** : other: Pre-dates OECD 482  
**Year** : 1986  
**GLP** : no data  
**Test substance** : other TS: 1,2-Dibromoethane obtained from Aldrich Chemical Company.  
**Method** : PRINCIPLE OF THE TEST METHOD: This method was developed to evaluate the effects of xenobiotics on DNA damage, quantitated as unscheduled DNA synthesis.

TEST COMPOUND CONCENTRATIONS USED: 10, 50 and 100 uM in DMSO (1%) in vitro.

CONTROL MATERIALS: DMSO used as vehicle control, and DMN (hepatocytes) and Methylmethanesulfonate (MMS) (spermatocytes) as positive controls.

TEST PERFORMANCE: In vitro: Hepatocytes and Spermatocytes were isolated and exposed for 18 hours to EDB and 10 uCi/ml 3H-thymidine. Exposure times were based on previous experience to maximize positive results in both spermatocytes and hepatocytes.

Hepatocytes were isolated and incubated for 4 hours in Williams Medium and 10 uCi/ml 3H-thymidine. Spermatocytes were isolated and incubated for 24 hours in Williams Medium and 10 uCi/ml 3H-thymidine. DNA repair was measured as unscheduled DNA synthesis (UDS) by quantitative autoradiography as net grains per nucleus (NG). A positive response in hepatocytes was an average >5 NG, and in spermatocytes it was >2NG in >10% of cells responding. The effects of pretreatment with metyrapone or diethylmaleate were included to evaluate the influence of hepatic mixed function oxidases (e.g. cytochrome P450) and glutathione, respectively, on EDB's genotoxicity.

**Result** : REPORT RESULTS: In vitro exposure to EDB caused a dose-dependent increase in DNA repair in both spermatocytes and hepatocytes.

CONCLUSION: The results demonstrated that the liver mixed function oxidases do not play a role in the production of genotoxic metabolites of EDB. EDB induction of UDS was decreased when liver glutathione was depleted. Thus, the authors conclude that the conjugation of EDB to glutathione may be the first step in the production of genotoxic metabolite in hepatocytes in vitro. The same pathway was suggested to be functioning in spermatocytes as well.

**Reliability** : (2) valid with restrictions

## 5. Toxicity

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19.12.2001

(16)

### 5.6 GENETIC TOXICITY 'IN VIVO'

Type : Dominant lethal assay  
Species : mouse  
Sex : male/female  
Strain : other: Male DBA/2J mice (9 weeks old) and C57BL/6J female mice (12-16 weeks old).  
Route of admin. : i.p.  
Exposure period : single dose  
Doses : 40 males received a single i.p. injection of 100 mg/kg of test material in physiologic saline.  
Result : negative  
Method : other: Consistent with OECD 478, and Epstein et. al. (1972).  
Year : 1992  
GLP : yes  
Test substance : other TS: 1,2-Dibromoethane  
Method : TEST ANIMALS USED: Male DBA/2J mice (9 weeks old) and C57BL/6J female mice (12-16 weeks old).

TEST COMPOUND CONCENTRATIONS USED: 40 males received a single i.p. injection of 100 mg/kg of test material in physiologic saline.

CONTROL MATERIALS: Positive control agent was procarbazine given as single i.p. injection of 400 mg/kg to 10 males. The solvent control used was physiologic saline.

TEST PERFORMANCE: Male mice were sequentially mated at 4-day intervals to 12-16 week old virgin females. Females were not examined for vaginal plugs. They were killed 12 days following removal from the breeding cage and examined. Each female was checked for pregnancy, total number of implants, early fetal deaths.

STATISTICS: The Mann-Whitney U Test was used for all results. Comparisons of pregnancy rates in treated groups and solvent control were made using the one-sided Fisher Exact test.

Result : REPORT RESULTS: The test material did not affect mating performance, the number of implants, live embryos or early and late embryonic deaths. The positive control, procarbazine, yielded the expected positive dominant lethal effects in both species.

Reliability : (2) valid with restrictions  
18.12.2001

(33)

Type : Dominant lethal assay  
Species : other: Rats and mice  
Sex : male/female  
Strain : other: SD Rats and BDF1 Mice  
Route of admin. : gavage  
Exposure period : 5 days  
Doses : Rat - 10 or 30 mg/kg; mice - 100 or 150 mg/kg. All doses administered via gastric intubation, dissolved in olive oil. All doses given once a day for 5 days.  
Result : negative  
Method : other: Not stated; pre-dates OECD 478  
Year : 1980  
GLP : no data  
Test substance : other TS: 1,2-Dibromoethane obtained from Tokyo Kasei Kogyo Company.  
Method : TEST ANIMALS USED: Male and female SD rats (12 weeks old) and BDF1 mice (10 weeks old).

## 5. Toxicity

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TEST COMPOUND CONCENTRATIONS USED: Rat - 10 or 30 mg/kg; mice - 100 or 150 mg/kg. All doses administered via gastric intubation, dissolved in olive oil. All doses given once a day for 5 days.

CONTROL MATERIALS: Positive control agent was ethyl methanesulfonate (EMS) given i.p.. The negative control used was olive oil given orally.

TEST PERFORMANCE: Rat: 15 male rats were given the test material once a day for 5 days, then mated overnight with an untreated virgin female in the pro-estrus stage. Females were checked in the morning for the presence of vaginal plug or sperm, and separated from males. Females were mated one per male and continued for 10 successive weeks. Mice: Groups of 7-9 male mice were given the test material once a day for 5 days, then mated with 2 untreated virgin females for 7 days. Females were examined every morning for the presence of a vaginal plug and caged separately when observed. Any females remaining in the male cage after one week were removed and replaced with 2 new females, and continued for 6 weeks. The mouse experiment was repeated twice. All pregnant rats and mice were killed 12-14 days after conception and the numbers of C.L., implants, live embryos and early and late embryonic deaths counted.

### Result

STATISTICS: The Mann-Whitney U Test was used for all results.  
: REPORT RESULTS: The test material did not affect mating performance in either species, and no adverse effects on number of implants, corpora lutea, live embryos or early and late embryonic deaths. The positive control, EMS, yielded the expected positive dominant lethal effects in both species.

### Reliability 19.12.2001

CONCLUSION: 1,2-dibromoethane was not mutagenic in the dominant lethal assay in rats and mice.  
: (3) invalid

(20)

### Type Species Sex Strain Route of admin. Exposure period Doses

: Drosophila SLRL test  
: Drosophila melanogaster  
: male/female  
: other: Berlin K Drosophila melanogaster adults  
:  
:  
: A single dose of 0.3 mM, administered in solution.

### Result Method Year GLP Test substance Method

: positive  
: other: Pre-dates OECD 477  
: 1974  
: no data  
: other TS: 1,2-Dibromoethane  
: TEST PERFORMANCE: The test material was administered as a solution to 2 adult males for 3 days, after which they were mated to two females for 3 days. A sequence of two 3-day breeding periods was initiated followed by one 4-day brood. The male was transferred to a new vial and remated with two new females after each breeding period.

### Result

: REPORT RESULTS: 1,2-dibromoethane was mutagenic in Drosophila producing the following lethals per number of chromosomes: 6/1201 (Brood 1), 18/1209 (Brood 2), and 16/1234 (Brood 3). Broods 2 and 3 represent the effect on spermatids and spermatocytes, respectively.

### Reliability 19.12.2001

: (3) invalid

(21)

## 5. Toxicity

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Type : Unscheduled DNA synthesis  
Species : mouse  
Sex : male  
Strain : Swiss Webster  
Route of admin. : i.p.  
Exposure period : single dose  
Doses : 0, 25, 50 and 75 mg/kg in DMSO. The volume was kept constant.  
Result :  
Method : other: Pre-dates OECD 486. Technique developed by Kohn, Erickson and Ewig (1976 and 1981). At present there is no international standardized test method for this assay.  
  
Year : 1981  
GLP : no data  
Test substance : other TS: 1,2-Dibromoethane obtained from Matheson, Coleman & Bell; redistilled before use.  
Method : TEST CELLS USED: Male Swiss Webster mice (CFW) weighing 25-35 grams. EDB, dissolved in DMSO, was administered via i.p. injection 3 hours prior to sacrifice in order to facilitate rapid absorption into the liver.

PRINCIPLE OF THE TEST METHOD: This method was developed to evaluate DNA strand breaks. The alkaline elution assay uses isolated hepatic nuclei and separates single-stranded DNA on the basis of its length. The small pore size filter eliminates the passage of long-stranded DNA and allows the examination of single-stranded DNA breaks caused by xenobiotics. The elution rate is directly related to the number of single-strand breaks, and increases as the number of breaks increases.

TEST COMPOUND CONCENTRATIONS USED: 0, 25, 50 and 75 mg/kg in DMSO. The volume was kept constant.

CONTROL MATERIALS: DMSO used as vehicle control, and X-irradiation of hepatic nuclei (DNA cross-link assay).

TEST PERFORMANCE: Hepatic nuclei (treated and untreated) were loaded onto filters, lysed with buffer (sodium lauryl sulfate, and sodium EDTA, pH7.4); pH was adjusted to 10 with tetraethylammonium hydroxide. A pump was hooked to the filter, the pH of the buffer adjusted to 12.3 and the DNA elution was pulled through the filter. Six 3 ml fractions were collected and amount of DNA quantitated. Results (elution rate constants) were analyzed using the Student's t-test.

Result : REPORT RESULTS: EDB caused a dose-dependent increase in the amount of damage to hepatic DNA, demonstrating the presence of alkali-labile sites in DNA.

CONCLUSION: It was not the intention to demonstrate whether or not EDB was mutagenic, but rather to examine whether bioactivation of EDB damages hepatic DNA by creating unstable adducts. The authors concluded that these results confirm previous efforts using sucrose density gradients that demonstrated the presence of DNA strand breaks after oral administration of EDB (75-220 mg/kg).

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(36)

Type : Unscheduled DNA synthesis  
Species : rat  
Sex : male  
Strain : other: CDF (F344)/CrIBr  
Route of admin. : other: i.p. and p.o.  
Exposure period :  
Doses : 10, 50 and 100 mg/kg in corn oil  
Result :  
Method : other: Pre-dates OECD 482.  
Year : 1986

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|                       |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-----------------------|---|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>GLP</b>            | : | no data                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Test substance</b> | : | other TS: 1,2-Dibromoethane obtained from Aldrich Chemical Company.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Method</b>         | : | <b>PRINCIPLE OF THE TEST METHOD:</b> This method was developed to evaluate the effects of xenobiotics on DNA damage, quantitated as unscheduled DNA synthesis.<br><br><b>TEST COMPOUND CONCENTRATIONS USED:</b> 10, 50 and 100 uM in DMSO (1%) in vitro; and 10, 50 and 100 mg/kg in corn oil in vivo (given i.p. and p.o).<br><br><b>CONTROL MATERIALS:</b> DMSO used as vehicle control, and DMN (hepatocytes) and Methylmethanesulfonate (MMS) (spermatocytes) as positive controls.<br><br><b>TEST PERFORMANCE:</b> In vivo: Rats were treated by gavage or i.p. injection with EDB in corn oil. Exposure times were based on previous experience to maximize positive results in both spermatocytes and hepatocytes.<br><br>Hepatocytes were isolated and incubated for 4 hours in Williams Medium and 10 uCi/ml 3H-thymidine. Spermatocytes were isolated and incubated for 24 hours in Williams Medium and 10 uCi/ml 3H-thymidine. DNA repair was measured as unscheduled DNA synthesis (UDS) by quantitative autoradiography as net grains per nucleus (NG). A positive response in hepatocytes was an average >5 NG, and in spermatocytes it was >2NG in >10% of cells responding. The effects of pretreatment with metyrapone or diethylmaleate were included to evaluate the influence of hepatic mixed function oxidases (e.g. cytochrome P450) and glutathione, respectively, on EDB's genotoxicity. |
| <b>Result</b>         | : | <b>REPORT RESULTS:</b> Following in vivo exposure to EDB, only hepatocytes exhibited UDS and then only when EDB was given by i.p. injection and only at the high dose. Spermatocytes did not show UDS after in vivo exposures.<br><br><b>CONCLUSION:</b> The results demonstrated that the liver mixed function oxidases do not play a role in the production of genotoxic metabolites of EDB. EDB induction of UDS was decreased when liver glutathione was depleted. Thus, the authors conclude that the conjugation of EDB to glutathione may be the first step in the production of genotoxic metabolite in hepatocytes. The same pathway was suggested to be functioning in spermatocytes as well.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Reliability</b>    | : | (2) valid with restrictions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 19.12.2001            |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| 18.12.2001            |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

(16)

### 5.7 CARCINOGENITY

### 5.8 TOXICITY TO REPRODUCTION

|                               |   |                                                                   |
|-------------------------------|---|-------------------------------------------------------------------|
| <b>Type</b>                   | : | Fertility                                                         |
| <b>Species</b>                | : | rat                                                               |
| <b>Sex</b>                    | : | male/female                                                       |
| <b>Strain</b>                 | : | other: Charles River CD rats                                      |
| <b>Route of admin.</b>        | : | inhalation                                                        |
| <b>Exposure period</b>        | : | 10 weeks (males) and 3 weeks (females)                            |
| <b>Frequency of treatment</b> | : | 7 hr/day, 5 days/week (males) and 7 hr/day, 7 days/week (females) |

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### Premating exposure period

Male : 10 weeks

Female : 3 weeks

Duration of test : 12 weeks for males and 4.5 weeks for females

Doses : 0, 19, 39 and 89 ppm (males) and 0, 20, 39, or 80 ppm (females)

Control group : yes

Method : other: Pre-dates OECD and US EPA Test Guidelines.

Year : 1979

GLP :

Test substance : other TS: Ethylene dibromide; purity 99%

Method : DURATION OF TEST: All females were sacrificed mid-gestation, calculated from the midweek of their presumptive mating. One group of males was killed after the 10 week exposure period. Another group was mated with females for 2 weeks and then killed.

DOSE LEVEL(s) and NUMBER OF DOSES: Four groups of males were exposed to 0, 19, 39 and 89 ppm (based upon time weighted average), administered 7 hr/day, 5 days/week for 10 weeks. Afterwards, 9-10 males/group were killed. An additional 9-10 males were housed individually with 2 females for mating for two weeks and then killed. In a separate part of the study, 4 groups of females were exposed to 0, 20, 39, or 80 ppm 7 hr/day, 7 days/week for 3 weeks, mated with males, and then killed at mid-gestation.

NUMBER OF ANIMALS/DOSE: The number of animals varied with each protocol. Generally, there were 30-33 males and 20 females/dose group. Pregnancy determined by daily inspection of vaginal smear for sperm.

VEHICLE: None.

INHALATION CHAMBER DESIGN: Rochester type stainless steel chamber with a volume of 3.5 cubic meters. Clean air flow rate of 10-12 changes per hour. EDB vapor was generated by bubbling nitrogen into a glass vessel that was maintained at 60 degrees C. EDB was mixed in the air stream in a plenum at the top of the chamber. EDB concentration measured 3 times/day and a time-weighted average calculated. Rats removed from inhalation chamber after each daily exposure. Rats had free access to food and water except during the 7 hour exposure period.

CAGING/HOUSING: Males exposed: Males mated with females were caged one male to two virgin females, and each male was mated with 4 females. Females exposed: After exposure, two females mated to one male and then females killed at mid-gestation.

CAGESIDE OBSERVATIONS: Animals were observed for mortality and body weight change and food consumption were measured.

STATISTICAL METHODS: Comparisons of analysis of variance, the Fisher's exact test and Mann-Whitney 2-tailed rank test were used to analyze the data.

GROSS PATHOLOGY: Males exposed for 10 weeks were killed, testes weighed and blood from the aorta obtained to measure serum testosterone. Males exposed and then mated with females for two weeks were killed and their reproductive organs removed and examined histologically. This included testes, epididymides, seminal vesicle, and prostate. Females mated to these males were killed at mid-gestation, and the number of implants, viable implants and resorptions determined. Females exposed and then mated with untreated males, were killed and the number of implants, viable implants, and resorptions determined. Also, their uterus and ovaries were examined histologically.

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**Result** : FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOEL, NOAEL):  
Observations: Males exposed to 39 and 89 ppm EDB had reduced body weight gain, and mortality was increased at 89 ppm (7/33). No adverse effects at 19 ppm. Females exposed to 80 ppm had reduced body weight gain and food consumption. Mortality was also increased at 80 ppm (10/50). No adverse effects at 20 and 39 ppm.

Reproductive performance: Males exposed to 89 ppm failed to impregnate any females. Females exposed to 80 ppm showed less frequent matings and pregnancies, with only 40% of the exposed rats becoming pregnant. There was no apparent effect on implants, resorptions or viable implants/dam. Females exposed to 80 ppm were in diestrus and did not begin to cycle until 3-4 days after exposure ended. This resulted in fewer females mating during the 10 week mating period.

Histological examination: Males exposed to 89 ppm displayed atrophy of the testes (10/10), epididymides (10/10), seminal vesicles (9/9) and prostate (10/10). There was also calcification of the testes and sperm granuloma in the epididymides. Marginal effects in the uterus and ovaries were observed in females exposed to 80 ppm (i.e. mild vacuolated degeneration of the epithelium of the uterus and ovarian cysts)

CONCLUSION: EDB had an adverse effect on reproductive performance at inhalation exposures of 89 ppm in males and 80 ppm in females. Males had reduced testicular weights and serum testosterone levels, atrophy of the reproductive organs and failed to impregnate any female rats. Females exposed to 80 ppm had abnormal estrus cycles during exposure. This returned to normal when exposure was terminated. Authors noted that the male protocol of 10 weeks exposure and 2 weeks mating was similar to a dominant lethal assay by Green et al. (1977), and did not cause dominant lethal mutations at 19 and 39 ppm.

**Reliability** : (2) valid with restrictions  
20.12.2001 (41)

**Type** : Fertility  
**Species** : rabbit  
**Sex** : male/female  
**Strain** : New Zealand white  
**Route of admin.** : s.c.  
**Exposure period** : 5 days  
**Frequency of treatment** : daily  
**Premating exposure period**  
**Male** :  
**Female** :  
**Duration of test** : 12 weeks  
**Doses** : 0, 15, 30, or 45 mg/kg body weight  
**Control group** : yes  
**Method** : other: There are no international test standards for this type of study.  
**Year** : 1991  
**GLP** : yes  
**Test substance** : other TS: Ethylene dibromide; purity 99%  
**Method** : SPECIES/SEX: Sexually mature noninbred New Zealand White rabbits. Mean age of 9 months when received and trained for 1-2 months prior to exposure. Mean weight was 4.3 kg. Virgin female rabbits were 5-7 months old.

ROUTE: Subcutaneous.

DURATION OF TEST: Dosed for 5 days, and then evaluated for up to 12 weeks post-dosing.

DOSE LEVEL(s) and NUMBER OF DOSES: EDB given in corn oil at 0, 15, 30, or 45 mg/kg body weight.

NUMBER OF ANIMALS/DOSE: 8-10 males/group for semen examination; 3 females/male/time point in the fertility study.

VEHICLE: Corn oil.

CAGING/HOUSING: Housed individually.

CAGESIDE OBSERVATIONS: Animals were observed for mortality, clinical signs and body weight change and food consumption.

STATISTICAL METHODS: Mixed-model analysis of variance used for all parameters.

TEST PROTOCOL: Sexually mature male rabbits were dosed subcutaneously. Weekly semen samples for 6 weeks pre-exposure, during the week of exposure and 12 weeks post-exposure were collected and analyzed. Analysis included: sperm concentration, number, morphology, viability, motion parameters, pH, osmolality, volume, fructose, citric acid, carnitine, protein, and acid phosphatase. Male fertility was assessed pre-exposure, at 4 weeks and 12 weeks post-exposure by artificial insemination of 3 females/male/time point. Time points were (1) pre-exposure, (2) 4 weeks post-exposure (for effects on spermatid) and 12 weeks post-exposure (for effects on fertility following complete spermatogenesis). There were 6 males per dose group. The percent pregnant, litter size, fetal body weight and structural development were examined.

CLINICAL CHEMISTRY: Blood samples were collected pre-exposure, 3 hrs, 3, 7 and 14 days post-exposure. Blood analyzed for: sorbitol dehydrogenase (SDH), alanine transaminase (ALT), total bile acids, cholesterol, protein, alkaline phosphatase (ALK), 5'-nucleotidase (NUC), creatine kinase (CK), albumin, urea nitrogen, and creatine.

GROSS PATHOLOGY: At termination of the study, rabbits were killed and target organs weighed and fixed in formalin (liver and kidney) or Bouin's fluid (testis and epididymides). Tissues were examined histologically.

#### Result

: FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOEL, NOAEL): 3 rabbits in the high dose group were killed in extremis on the last day of dosing. Rabbits in the high dose exhibited hunched posture, lethargy, rough coat and hypothermia. Food consumption was decreased in a dose-related manner in all dose groups, but there were not body weight reductions. Changes in clinical chemistry measurements were evident in the high dose group only, in practically all endpoints measured (most were elevated above normal values). Of the 13 parameters measured to assess the effects on sperm, only 3 were adversely effected by treatment with EDB: motion characteristics (curvilinear and straight line velocities), ALH (amplitude of lateral head displacement), and percentage of motile sperm. All three parameters were decreased in comparison to controls. Accessory gland function was examined in 11 parameters which were measured in rabbit semen. Of these, 4 were adversely effected: pH, ejaculate volume, total and tartrate-resistant AP activities. Male fertility was not effected by treatment as determined by the percentage of pregnant females or mean litter size. The only apparent effect was a decrease in the mean fetal body weight when adjusted for litter size at 4 weeks post-dosing in high dose males rabbits. No increase in anomalies were determined. Histologically there was liver and bile duct damage (hepatocyte necrosis and bile duct proliferation/hyperplasia) in high dose males. The authors concluded that EDB has an adverse effect on sperm which is evident at doses which also



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caused 30% mortality and liver damage in 43% of the survivors. The authors suggest that the effects on sperm are at the level of the epididymis (epididymal spermatozoa, late spermatids, and increased AP activity in seminal plasma).

**CONCLUSION:** This study was conducted to assess the male reproductive effects of EDB in the rabbit in an effort to evaluate the rabbit as suitable animal model for human response. The rabbit was not as sensitive as humans, based upon other experimental evidence, but is considered an acceptable animal model for human response to EDB exposure since semen quality is altered in both species.

**Reliability**  
19.12.2001

: (2) valid with restrictions

(24)

### 5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

|                               |   |                                                                                                                         |
|-------------------------------|---|-------------------------------------------------------------------------------------------------------------------------|
| <b>Species</b>                | : | rat                                                                                                                     |
| <b>Sex</b>                    | : | male/female                                                                                                             |
| <b>Strain</b>                 | : | other: Charles River CD                                                                                                 |
| <b>Route of admin.</b>        | : | inhalation                                                                                                              |
| <b>Exposure period</b>        | : | Days 6-15 of gestation                                                                                                  |
| <b>Frequency of treatment</b> | : | 23 hrs per day for 10 consecutive days                                                                                  |
| <b>Duration of test</b>       | : | Day 20 of gestation                                                                                                     |
| <b>Doses</b>                  | : | 0, 20, 38 and 80 ppm (based upon time weighted average),                                                                |
| <b>Control group</b>          | : | yes                                                                                                                     |
| <b>Method</b>                 | : | other: Pre-dates OECD and US EPA Test Guidelines.                                                                       |
| <b>Year</b>                   | : | 1977                                                                                                                    |
| <b>GLP</b>                    | : |                                                                                                                         |
| <b>Test substance</b>         | : | other TS: Ethylene dibromide; purity 99%; obtained from Aldrich Chemical Company.                                       |
| <b>Method</b>                 | : | SPECIES/SEX: Charles River CD rats; females were nulliparous; males of the same strain and source were used for mating. |

AGE at Start of Test: Sexually mature females, age not specified.

ROUTE: Inhalation.

DURATION OF TEST: All females were sacrificed on day 20 of gestation.

DOSE LEVEL(s) and NUMBER OF DOSES: 0, 20, 38 and 80 ppm (based upon time weighted average), administered on days 6 thru 15 of gestation; 23 hrs per day for 10 consecutive days. Two control were used, one with unrestricted food and one with restricted access to food.

NUMBER OF ANIMALS/DOSE: 15-17F per dose. Pregnancy determined by daily inspection by vaginal smear for sperm.

VEHICLE: None.

**INHALATION CHAMBER DESIGN:** Rochester type stainless steel chamber with a volume of 3.5 cubic meters. Clean air flow rate of 10-12 changes per hour. EDB vapor was generated by bubbling nitrogen into a glass vessel that was maintained at 30 degrees C. EDB was mixed in the air stream in a plenum at the top of the chamber. EDB concentration measured every 2 hours during exposure. During the 10 day inhalation period, EDB-treated rats were housed in the inhalation chamber. The feed was changed daily to prevent possible accumulation of EDB in the feed.

**CAGING/HOUSING:** Each female was paired with one male for mating.

After confirmation of mating, females were returned to their cage.

**CAGESIDE OBSERVATIONS:** Observations were made of maternal welfare and fetal development. Body weight and feed consumption were measured during and after the exposure period.

**STATISTICAL METHODS:** The Fisher exact test and Mann-Whitney 2-tailed rank test were used to analyze the data.

**GROSS PATHOLOGY:** Females were sacrificed on day 20 and the abdominal and thoracic cavities were examined. The umbilical cord was clamped and severed distally in order to prevent blood loss. Fetuses were removed from the uterine horn, weighed and examined for external anomalies. One half of the viable fetuses were fixed in Bouin's solution and examined for soft tissue anomalies by free-hand slicing method. The remaining viable fetuses were fixed in 70% alcohol, eviscerated, stored in 1% KOH, stained in alizarin red, and the skeletons examined for anomalies.

**FERTILITY AND REPRODUCTIVE PERFORMANCE:** The following data were recorded for each group.

o numbers of resorptions (early and late), and viable and dead fetuses.

o number of pregnant females (based upon presence of implantation sites at autopsy.

o number of live fetuses/dam

o fetal weight

#### Result

: **FINDINGS/MEASURED ENDPOINT/INDEX** (i.e. LOEL, NOAEL): Deaths were reported at 80 ppm. Weight loss was evident at 38 and 80 ppm. Feed consumption was reduced at all EDB concentrations and remained depressed at 80 ppm after termination of exposure. There were a reduced number of implants and increased resorptions at 80 ppm. Body weight of fetuses was reduced at 38 ppm. Since there were no fetuses at 80 ppm, this endpoint could not be determined. There were no external soft tissue or skeletal anomalies observed that were of concern. A Maternal NOAEL in this developmental toxicity test was not demonstrated, owing to the decreased body weight gain and feed consumption during exposure at 20 ppm. An NOAEL for fetal effects was demonstrated at 20 ppm, with decreased number of viable fetuses and increased resorptions at 38 ppm.

**CONCLUSION:** EDB, via inhalation, had an adverse effect on maternal welfare, measured by decreased body weight gain, feed consumption, and survival. There were no anomalies observed in the soft tissue or skeletal tissue.

**Reliability**  
18.12.2001

: (2) valid with restrictions

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**Species**  
**Sex**  
**Strain**  
**Route of admin.**  
**Exposure period**  
**Frequency of treatment**  
**Duration of test**  
**Doses**  
**Control group**  
**Method**  
**Year**  
**GLP**

: mouse  
: male/female  
: CD-1  
: inhalation  
: Days 6-15 of gestation  
: 23 hrs per day for 10 consecutive days  
: Day 18 of gestation  
: 0, 20, 38 and 80 ppm (based upon time weighted average)  
: yes  
: other: Pre-dates OECD and US EPA Test Guidelines.  
: 1977  
:

## 5. Toxicity

Id 106-93-4

Date 28.12.2001

**Test substance** : other TS: : Ethylene dibromide; purity 99%; obtained from Aldrich Chemical Company.  
**Method** : SPECIES/SEX: CD-1 mice; females were nulliparous; males of the same strain and source were used for mating.

AGE AT START OF TEST: Sexually mature females, age not specified.

ROUTE: Inhalation.

DURATION OF TEST: All females were sacrificed on day 18 of gestation.

DOSE LEVEL(s) and NUMBER OF DOSES: 0, 20, 38 and 80 ppm (based upon time weighted average), administered on days 6 thru 15 of gestation; 23 hrs per day for 10 consecutive days. Two control groups were used, one with unrestricted food and one with restricted access to food.

NUMBER OF ANIMALS/DOSE: 18-22F per dose. Pregnancy determined by daily inspection for presence of copulation plugs.

VEHICLE: None.

INHALATION CHAMBER DESIGN: Rochester type stainless steel chamber with a volume of 3.5 cubic meters. Clean air flow rate of 10-12 changes per hour. EDB vapor was generated by bubbling nitrogen into a glass vessel that was maintained at 30 degrees C. EDB was mixed in the air stream in a plenum at the top of the chamber. EDB concentration measured every 2 hours during exposure. During the 10 day inhalation period, EDB-treated mice were housed in the inhalation chamber. The feed was changed daily to prevent possible accumulation of EDB in the feed.

CAGING/HOUSING: Each female was exposed to proven male breeders. After confirmation of mating, females were returned to their cage.

CAGESIDE OBSERVATIONS: Observations were made of maternal welfare and fetal development. Body weight and feed consumption were measured during and after the exposure period.

STATISTICAL METHODS: The Fisher exact test and Mann-Whitney 2-tailed rank test were used to analyze the data.

GROSS PATHOLOGY: Females were sacrificed on day 18 and the abdominal and thoracic cavities were examined. The umbilical cord was clamped and severed distally in order to prevent blood loss. Fetuses were removed from the uterine horn, weighed and examined for external anomalies. One half of the viable fetuses were fixed in Bouin's solution and examined for soft tissue anomalies by free-hand slicing method. The remaining viable fetuses were fixed in 70% alcohol, eviscerated, stored in 1% KOH, stained in alizarin red, and the skeletons examined for anomalies.

FERTILITY AND REPRODUCTIVE PERFORMANCE: The following data were recorded for each group.

o numbers of resorptions (early and late), and viable and dead fetuses.

o number of pregnant females (based upon presence of implantation sites at autopsy.

o number of live fetuses/dam

o fetal weight

**Result**

: FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOEL, NOAEL): Deaths were reported at 38 and 80 ppm, and also in the control group on a restricted diet. It was reported the control deaths were due to cannibalism, which means they were grouped housed. Weight loss was evident at 20 and 38 ppm (there were no animals alive in the 80 ppm group). There were reduced fetal body weights and increased late resorptions at 20 ppm. At 38 ppm there were reduced numbers of viable fetuses, increased resorptions and reduced fetal body weight. In the restricted feed control groups there were reduced viable fetuses and increased resorptions. Since there were no fetuses at 80 ppm, these endpoints could not be determined. There were 3/218 fetuses with exencephally at 20 ppm. The number of runts was increased at 38 ppm. The number of skeletal anomalies observed was increased in the 20 and 38 ppm groups. These ranged from incomplete ossifications to unossified skeletal tissue. A NOAEL in this developmental toxicity test was not demonstrated.

CONCLUSION: EDB, via inhalation, had an adverse effect on maternal welfare, measured by decreased body weight gain, feed consumption, and survival. There were increased incidences of skeletal anomalies observed in the mice exposed to EDB.

**Reliability**  
20.12.2001

: (2) valid with restrictions

(43)

**5.10 OTHER RELEVANT INFORMATION****5.11 EXPERIENCE WITH HUMAN EXPOSURE**

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### 7.1 END POINT SUMMARY

### 7.2 HAZARD SUMMARY

### 7.3 RISK ASSESSMENT